

THE AMERICAN JOURNAL
OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

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NUMBER 1

HERPETIC INFECTION OF THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO *

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Nashville, Tenn.)*

To date attempts to infect chicks and chickens with the virus of herpes simplex have been unsuccessful. Recent work in this laboratory has proved the susceptibility of the extra-embryonic membranes of the chick to the viruses of fowl-pox, cow-pox and herpes simplex.¹ This paper is a more detailed account of the experiments dealing with herpes simplex.

Fertile white leghorn eggs are incubated in the usual manner for 12 to 14 days. The eggs are then candled to locate the embryo and air sac. Each of these is ringed with a colored pencil. The surface of the shell overlying the embryo is carefully cleansed with green soap. By means of a cutting carborundum disc attached by a flexible shaft to a small electric motor a square window 1 to 1.5 cm. in diameter is cut in the shell over the embryo. The window is then coated with a thin layer of paraffin and gently stripped from the underlying shell membrane. Exercising diligent aseptic precautions the underlying shell membrane is removed by cutting around the edges of the window with blunt pointed scissors. This exposes the extra-embryonic membranes, the chorion being uppermost. This membrane may then be easily inoculated. By means of a transfusion syringe filled with a mixture of sterile paraffin and vaseline the edges of the window are ringed, a sterile coverslip is placed over the opening and the edges sealed with a hot needle.

The herpetic virus used in this work was the HF strain obtained from the Rockefeller Institute. Glycerinated brain was emulsified

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and inoculated intracerebrally into a rabbit which was killed 4 days after developing a typical herpetic encephalitis. The brain was removed aseptically and preserved fresh in the ice-box, as well as in glycerin. Small fragments of fresh brain (approximately 1 mm. cubes) were used for inoculation. Transplants were made after 48 hours by removing the membrane sterily and introducing fragments of the infected membrane into other eggs. It was found that this method was rather tedious, consequently an attempt was made to inoculate eggs by using ground, emulsified brain and to transplant by inoculating ground, emulsified membranes, with the result that more abundant infections developed. The virus has been carried through six generations in the chick membranes.

In experiments using tissue fragments for inoculation changes may be noted in the chorio-allantoic membranes within 12 hours. The first change is a congestion of the vessels. Within 24 hours one sees around the bit of inoculum a rather opaque, grayish red zone which spreads peripherally. Careful examination at this time reveals within and around this zone minute grayish opacities just visible at a magnification of 30 diameters. These are particularly abundant along the course of the blood vessels. At 36 hours well defined, raised, grayish opacities, some of which are more than 1 mm. in diameter, are visible to the naked eye. As the lesion progresses these smaller areas tend to coalesce so that at the end of 72 to 96 hours the whole of the exposed membrane may be involved. Inoculation of ground, emulsified material leads to similar lesions. This method, however, provokes a more diffuse reaction which develops more rapidly. By either method the lesions are focal in distribution.

In this work some of the embryos die, due presumably to trauma. A large percentage of those that survived the inoculation were sacrificed at 2, 3, or 4 days for histological study and for further transfers. A few were allowed to run their course undisturbed. In some the process involved the membranes extensively and led to the death of the embryo; in others the involvement was less extensive and the chick hatched in the usual manner. Such chicks showed no gross changes. Examination of embryos which died also showed no changes. At other times the lesions developed in the usual manner and then regressed, ending in complete gross resolution. One egg which showed typical gross lesions with subsequent complete resolution was reinoculated, resulting in another characteristic "take."

For microscopic study the membranes were fixed in Zenker's solution and stained with hematoxylin and eosin or with eosin and methylene blue. The vessels throughout the entire membrane appear congested and there is some hemorrhage. There is a marked polymorphonuclear leukocytic reaction beneath the ectoderm of the chorion. In addition one sees large mononuclear leukocytes scattered here and there. In older lesions, particularly those in which tissue fragments served as the inoculum, there are many fibroblasts. In one instance there is a marked hyperplasia of ectoderm with a minimal amount of necrosis. In another, necrosis is the important feature and there is little or no hyperplasia. In any event some of the cells which remain show the characteristic intranuclear changes of infection due to the virus of herpes simplex.

Within the nuclei of ectodermal cells one sees inclusions of different size, homogeneity and staining reaction. This type of change is usually seen in groups of cells which correspond to the focal lesions noted in the gross, but it may be observed in isolated cells. The nucleus is almost invariably swollen and the chromatic material is collected along the nuclear membrane. Within such cells one sees usually one, but sometimes two or more intranuclear masses distinct from the nucleoli. Some of these bodies are quite small, no larger than nucleoli, others are so large that they almost completely fill the greatly swollen nuclei. They are separated from the nuclear membrane by a clear zone, and are acidophilic in reaction. The smaller bodies appear to be composed of minute granules, while the larger ones are more homogeneous and hyaline in appearance. The largest inclusions stain basophilically and under high magnification appear to be composed of closely packed, minute, basophilic granules. Within other nuclei the inclusion may completely fill the nuclear space. These inclusions stain faintly and appear very finely granular. Such nuclei may become exceedingly large, sometimes attaining a volume considerably greater than that of the entire normal cell. In these nuclei there is a delicate bluish reticulum which appears to divide the nucleus into many small compartments. These compartments are filled with minute, almost invisible, lavender granules. The cytoplasm of these cells shows few if any changes. Occasionally it appears foamy, but this is not the rule.

DISCUSSION

It is of considerable interest that one is able to infect the chorio-allantoic membranes of white leghorn chick embryos with a strain of herpes simplex virus which is innocuous to adult white leghorn chickens both by corneal and by cutaneous inoculation. The gross lesions of the herpetic membranes are entirely comparable to those of the herpetic keratitis of rabbits. Specific intranuclear inclusions are seen in the cells of the infected membranes. Both eosinophilic and basophilic inclusions are present and are characteristic of the herpetic inclusions observed in other experimental animals. The peculiar reticulum and minute lavender granules within many of the swollen nuclei constitute a very interesting change somewhat suggestive of that described by Goodpasture and King,² and again by Goodpasture and Woodruff,³ in the cytoplasm of epithelial cells within the lesions of molluscum contagiosum. It has not been possible, however, to demonstrate any "elementary corpuscles" similar to those described by Borrel in fowl-pox, by Lipschütz in molluscum contagiosum, and by Paschen in vaccinia.

It has been suggested by Goodpasture and his coworkers¹ that the method of inoculating chick embryos may be of value in the study of various other virus diseases, including those which have not been engrafted upon a foreign host. The fact that the embryonic membranes of the chick are more susceptible than baby or adult chicks points to such a conclusion.

CONCLUSIONS

1. The chorio-allantoic membranes of the chick embryo are susceptible to infection with a strain of herpes simplex virus which is innocuous to adult chickens of the same breed.

2. The microscopic lesions of these membranes are like those of herpetic lesions of mammals.

3. A peculiar nuclear change in ectodermal cells is described, characterized by enormous enlargement of the nucleus, and by a partitioning of it by delicate trabeculae into compartments which are filled by minute, uniform and faintly stained basophilic granules.

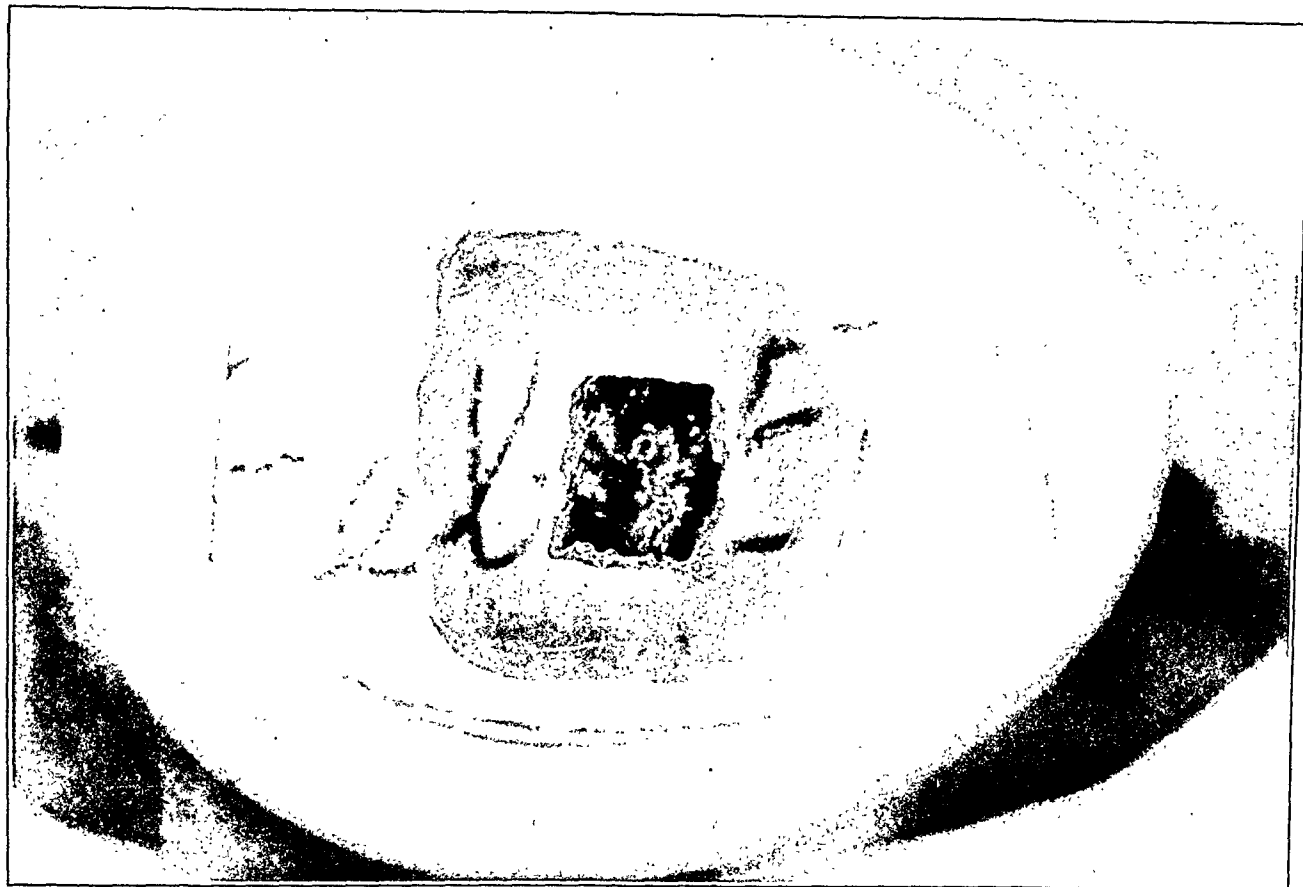
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2. Goodpasture, E. W., and King, Howard. A cytologic study of molluscum contagiosum. *Am. J. Path.*, 1927, 3, 385.
3. Goodpasture, E. W., and Woodruff, C. Eugene. A comparison of the inclusion bodies of fowl-pox and molluscum contagiosum. *Am. J. Path.*, 1931, 7, 1.

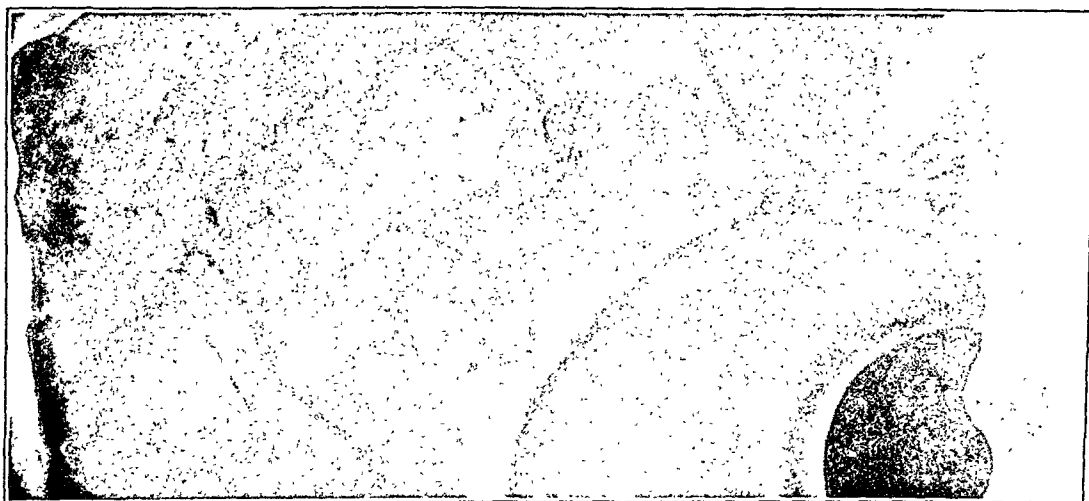
DESCRIPTION OF PLATES

PLATE I

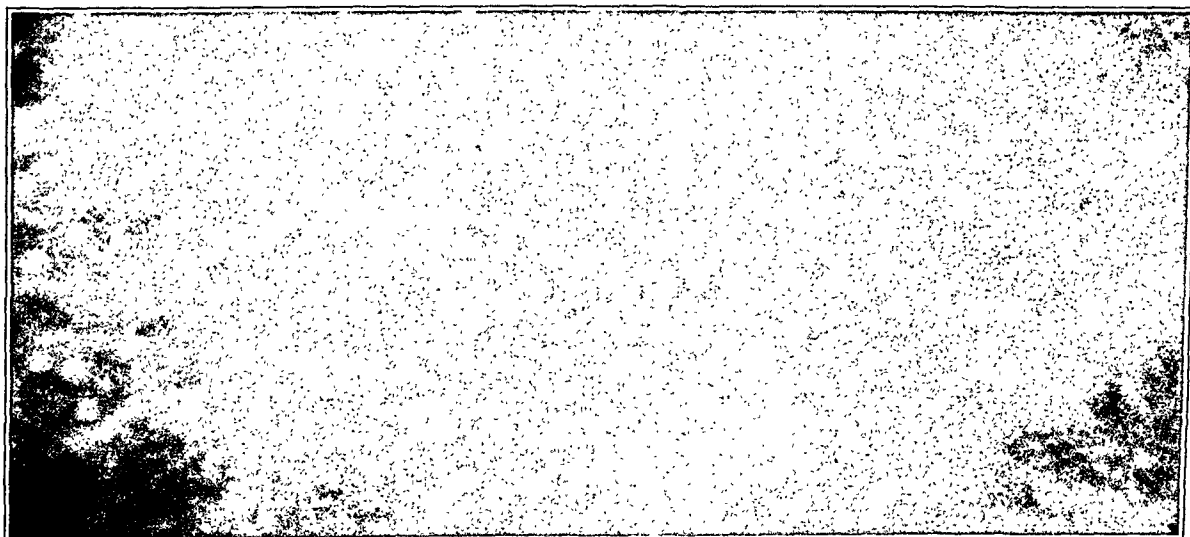
- FIG. 1. Gross photograph showing the glistening pox on the chorio-allantoic membrane of a 48 hour inoculated egg. $\times 1.5$.
- FIG. 2. Photograph of portion of above membrane magnified eight times.
- FIG. 3. Photomicrograph of 36 hour membrane showing the diffuse, small, pox-like eruptions. $\times 10$.



I



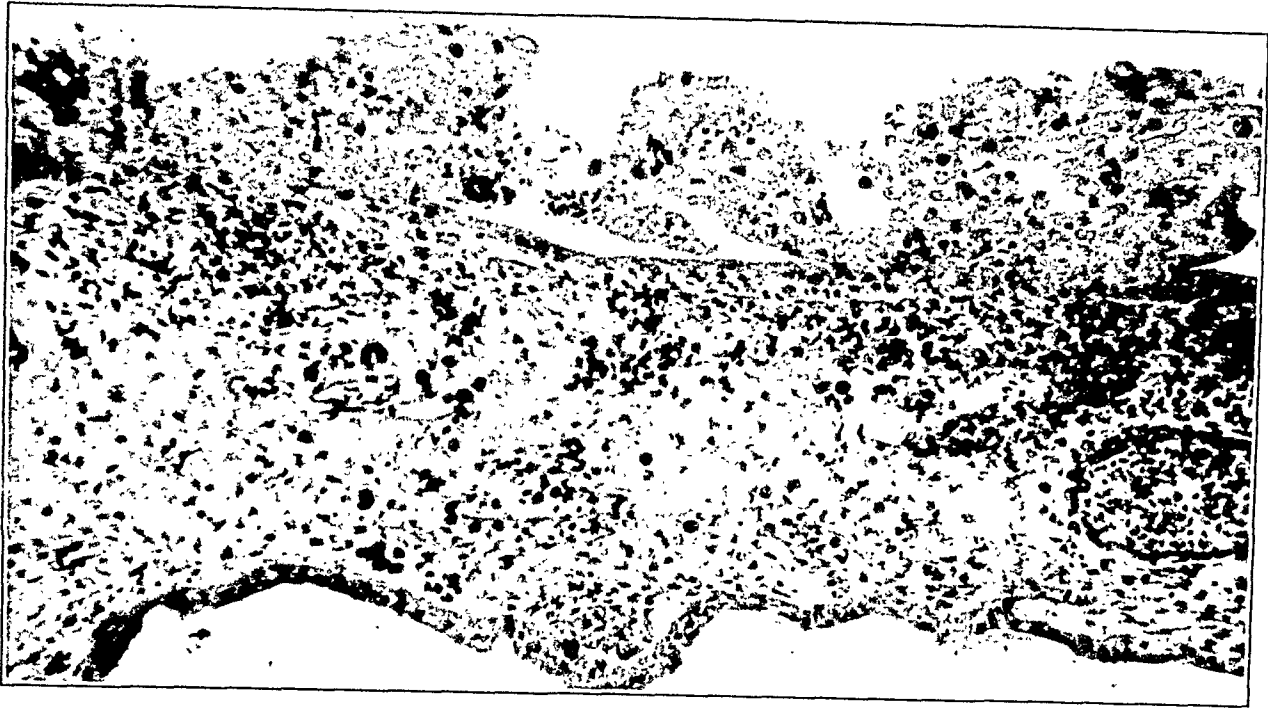
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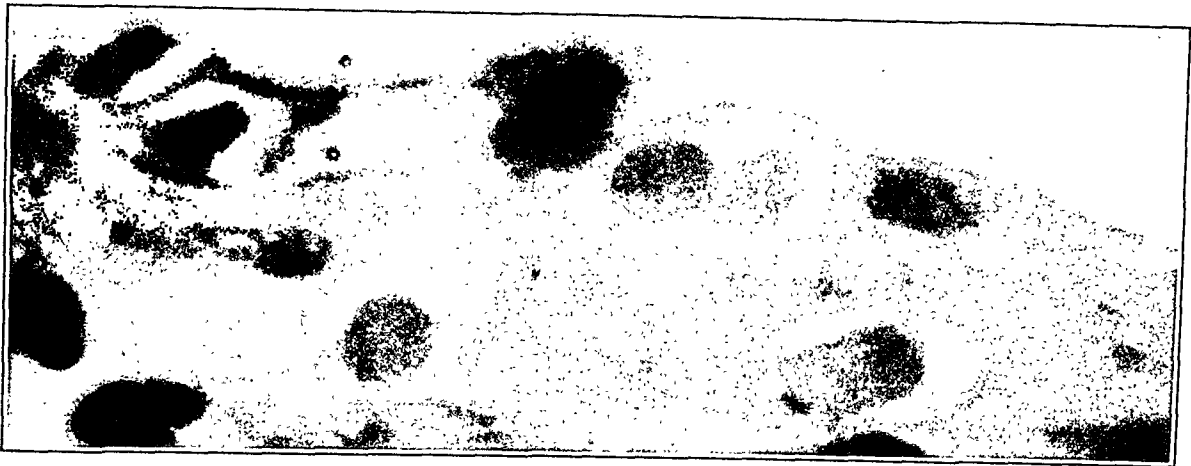
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PLATE 2

- FIG. 4. Photomicrograph of entire chorio-allantoic membrane at the site of a hyperplastic lesion. Inclusion bodies may be seen within the nuclei of epithelial cells. The inflammatory exudate beneath the lesion is also illustrated. Eosin-methylene blue stain. $\times 200$.
- FIG. 5. Ectoderm of chorion showing several large, very finely granular inclusions which completely fill the nuclei. Eosin-methylene blue. $\times 1800$.
- FIG. 6. Ectoderm of chorion showing several smaller intranuclear inclusions which appear granular. Eosin-methylene blue. $\times 1800$.
- FIG. 7. Two large ectodermal cells from the hyperplastic chorion. The one on the right shows a poorly defined cytoplasm represented by the clear zone around the greatly enlarged nucleus. The dark staining reticulum is illustrated. The finely granular material can be seen within the compartments formed by the interlacing trabeculae. The cell on the right shows the finely granular appearance of the large inclusions without the reticular material. Eosin-methylene blue. $\times 2300$.
- FIG. 8. Two intranuclear inclusions in the ectodermal cells of the chorion, similar to those in Fig. 2. Eosin-methylene blue. $\times 1800$.



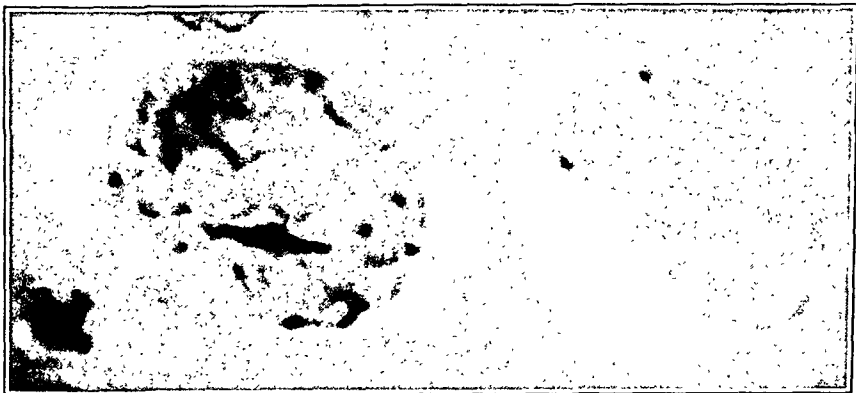
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CELLULAR INCLUSIONS IN CEREBRAL LESIONS OF LETHARGIC ENCEPHALITIS *

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In many of the articles dealing with encephalitis lethargica the previous work on the subject has been reviewed. Consequently no attempt will be made here to discuss the literature. Excellent summaries may be found in the papers of Zinsser,¹ von Economo,² and in the report of the Matheson Commission.³

Many investigators have implicated a filterable virus as the etiological factor in encephalitis lethargica, and some have claimed success in transmitting an encephalitis to experimental animals by the inoculation of brain or nasopharyngeal washings. In every instance, except one in which an encephalitis has been transmitted in series in experimental animals, the virus has been proved identical with that of herpes simplex. In the one exception the virus was that of rabies. Only nine strains of herpetic virus have been isolated from human encephalitis.³ It has been the experience of most workers that repeated inoculations of material from encephalitic patients have uniformly led to negative results. Furthermore, Flexner and Amoss⁴ have isolated a strain of herpes virus from the spinal fluid of a luetic patient in whom they found evidence neither of herpes nor of encephalitis. From the numerous negative results which have been published, and the large number which undoubtedly have not been published, it must be concluded that the isolation of a virus from cases of lethargic encephalitis infectious for laboratory animals is, at least, an unusual occurrence. The association of herpetic virus with human encephalitis seems, on the basis of available evidence, adventitious.

At the present time, diseases having filterable viruses as etiological agents are for the most part characterized by certain cytological changes in their specific lesions. These may be in the form of inclusion bodies within the nucleus or the cytoplasm, hyperplasia of cells

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or simply necrosis of individual cells. In spite of the fact that herpes simplex is characterized by definite intranuclear inclusion bodies, the workers who have implicated this virus in the etiology of human encephalitis have never demonstrated in human cases inclusions characteristic of herpetic infections. Herzog,⁵ Da Fano,⁶ and others have described vague intracellular bodies which are not at all typical of virus inclusions.

It is the purpose of this paper to present a case of human encephalitis, clinically encephalitis lethargica, in which intranuclear inclusion bodies were found. These inclusions are quite definite, and while they simulate in certain respects the inclusions associated with herpetic infections, there are certain morphological differences. Furthermore inoculations of brain tissue upon the cornea and intracerebrally in rabbits failed in every case to induce herpetic infection.

REPORT OF CASE

Clinical History: The patient, W. W., white, age 16 years, was brought to the Out-Patient Department July 18, 1931. His mother, who brought him to the clinic and gave the history, said that he had had a sunstroke. Additional points in the history were obtained by a social service worker.

Sixteen months ago the patient was found on the street in an unconscious condition and was taken home. No other details could be obtained. Twelve months ago, while attending a boys' camp, he is said to have had a sunstroke. He was taken to a hospital which recorded the presence of headache, sore throat, a diffuse rash over the body, temperature of 99° F, and clear spinal fluid under normal pressure with 14 cells. He recovered from this attack rapidly.

Eight months ago he had a light attack of influenza, but did not go to bed and had no doctor. Two weeks ago while working in the sun with his father he suddenly said, "I feel just like cussing you." A few nights later his sister came home and said that the patient was in a park, acting queerly and staring at trees. About this time he started walking about at night, refused to undress, was quite nervous and smoked a great deal. One week before admission he began having involuntary jerking movements of the arms and legs. Other information obtained without definite dates of onset was that he had gradually become very slow and deliberate in his movements and his memory had failed considerably. He was referred to as "the boy who slept standing up" by one of his recent employers. For some time he had had diplopia.

On admission to the Vanderbilt Hospital his temperature was 99° F, pulse 32, respirations 20. He was well developed and nourished. Voluntary movements and speech were slow and deliberate. The face had a typical mask appearance. During the examination he got out of bed several times and walked around the room. In walking the head was bent slightly forward. Occasionally there was an involuntary, convulsive jerking of the arms and legs. The head presented nothing of interest. The pupils were equal, regular and round. They reacted normally. The arms offered some rigidity to passive motion. The superficial

and deep reflexes were equal and hyperactive. There were no pathological reflexes. The neck was moderately stiff. There was a slight, fine tremor of the fingers. One observer noted the presence of "herpes" on July 29, eleven days after admission. How long these lesions had been present was not stated.

Laboratory Findings on Admission: Blood: White blood cells 6200, 73 per cent polymorphonuclear leukocytes, 24 per cent lymphocytes. Hemoglobin 14.5 gm. Wassermann negative.

Urine: Normal.

Spinal Fluid: Slightly increased pressure, clear, 14 cells, sugar 58 mg., globulin positive. Wassermann negative.

Course in Hospital: While in the hospital little was done for the patient with the exception of sedatives and forced feedings. During the first week the temperature chart showed variations from 98.6 to 100.8° F, usually reaching its peak at 4.00 or 8.00 P.M. Toward the end of the second week it reached 101.8° F. For two weeks it ranged from 99.6 to 102°; following this it showed fairly marked fluctuation with a gradual tendency to rise higher each day until he died on August 31. At this time his temperature was 106° F. His course was progressively down hill. He became more and more lethargic, and the convulsions and twitchings increased in severity and frequency. His expression became typically masked and he exhibited a definite lead-pipe rigidity of the extremities. Toward the end of his illness the convulsive twitchings became almost constant. Repeated examination of the spinal fluid showed little change except for a slight decrease of sugar and number of cells. Guinea pig inoculations and smears of spinal fluid were repeatedly negative.

Pathological Anatomy: Grossly, the brain showed no changes of a specific nature. The meninges were smooth, of normal thickness and glistening. There was no demonstrable increase in the amount of cerebrospinal fluid, nor was this fluid cloudy or blood-tinged. The vessels within the subarachnoidal space were definitely congested. Transverse sections through the brain showed a diffuse congestion and some edema. There were no definite areas of hemorrhage or necrosis.

MICROSCOPIC EXAMINATION

Microscopically, numerous changes are seen. These changes fall into two definite groups, the one affecting the blood vessels, the other individual cells. The sections studied were taken from all of the important cerebral structures, the spinal cord and the cerebellum.

The lesions associated with the blood vessels are congestion, hemorrhage and a lymphocytic infiltration of the adventitial coat of the smaller arteries and veins. These changes are seen in all the sections studied, with the exception of those taken from the cerebellum and spinal cord. In spite of their wide distribution these

changes are most evident in the sections from the cortex, the lenticular, caudate and red nuclei, and the thalamus. Examples of these changes are seen in the white as well as the gray matter. The congestion is the most constant finding, minute areas of hemorrhage and perivascular infiltration being somewhat less common. The areas of hemorrhage are always quite small and are seen around the capillaries chiefly. The cellular infiltration is composed almost entirely of lymphocytes. An occasional plasma cell is seen, but there are no polymorphonuclear leukocytes. While this exudate is rather scanty it is nevertheless quite definite. It is not a perivascular infiltration in the sense that the cells lie within the perivascular space. Close examination shows them to be enmeshed by the fibers of the vessel wall, particularly the adventitial sheaths. The smaller arteries are the vessels chiefly affected. No thrombi are seen within any of the blood vessels.

The changes affecting the cells are of four types. In eosin-methylene blue preparations they may appear in the form of eosinophilic intranuclear bodies entirely separate from the nucleolus of the cell, with or without irregular eosinophilic cytoplasmic bodies, or as simple degeneration and necrosis, or as phagocytosis of such cells. These changes are found in all parts of the cerebrum, but are most obvious in the left motor cortex, the red nucleus and the caudate nucleus. The intranuclear bodies may appear without any accompanying cytoplasmic bodies or any detectable degenerative changes. The intracytoplasmic masses are not seen in cells which do not show the intranuclear bodies and are almost invariably accompanied by degenerative changes of various types within the cell. Other cells may undergo degenerative changes without exhibiting bodies within the nucleus or the cytoplasm.

The intranuclear masses occur chiefly in the large ganglion cells, but are also seen rarely in neuroglial cells. There may be one or several within the nucleus, usually only one. They vary in size from those which are perhaps twice as large as the nucleolus to some which completely fill the nucleus. They may be round, oval or horseshoe-shaped. Associated with this variation in size there is a corresponding variation in appearance. The smaller bodies tend to be more eosinophilic and present a more granular appearance. The larger bodies are more homogeneous and in most instances tend to have a lavender color. In affected cells the chromatic material of the nu-

cleus is concentrated along the nuclear membrane. These intranuclear bodies are entirely separate and easily distinguishable from the nucleoli.

The cytoplasmic bodies occur only in ganglion cells. They are less definite in their appearance, are usually multiple, and vary in size from very small rounded masses to those which almost fill the cytoplasm. They are correspondingly irregular in shape. Their staining reaction is much more uniform. They appear as homogeneous, hyaline, pink-staining masses. As to distinctness of outline they again vary considerably. Many are rather sharply defined, but equally as many merge almost imperceptibly with the cytoplasm of the cells. In only a few instances are they surrounded by a halo.

The degenerative changes are frequently associated with the bodies just described. In many instances, however, one sees degenerating cells which are not so involved. Surrounding such cells one frequently sees accumulations of apparently healthy cells which vary in shape from round to spindle. These cells have rather scanty cytoplasm which stains lavender. Their nuclei are large as compared to the cytoplasm. Occasionally, one sees a necrotic ganglion cell which has been invaded by one of these cells. Such lesions are interpreted as pseudo- and true neuronophagia respectively. The nature of the phagocytic cells, whether neuroglial or microglial in origin, is unknown.

What appears as a later stage of the process of phagocytosis is the accumulation of cells, resembling the phagocytic cells, which are occasionally seen unassociated with degenerating or necrotic nerve cells. These phagocytic cells appear as rosettes. In some instances one is able to see small purplish fragments of material which resemble calcium in the center of such a rosette. These changes are seen most commonly in the pons, the lenticular and the caudate nuclei.

In addition to the changes found in the nervous system there was some bronchopneumonia, bilateral fibrinous pleuritis, and a few petechiae within the mucosa of the gastro-intestinal tract.

DISCUSSION

The inclusion bodies found within the nerve cells and neuroglial cells are comparable morphologically to those seen in the lesions of herpes simplex, zona, varicella and virus III disease of rabbits. Of

these four types they resemble the inclusions seen in herpes simplex most closely. There are, however, several differences. The inclusions seen in this case never appear as extremely large, finely granular, lavender bodies which completely fill the nucleus. In herpetic lesions the intracytoplasmic masses are not seen. The inclusions in this case are more numerous in the cortex and are accompanied by a minimal amount of necrosis, while in herpetic encephalitis of rabbits the brain stem and mesencephalon are chiefly involved and there is widespread necrosis of nerve cells. One is able to conclude quite definitely that the cellular inclusions are not associated with the virus of herpes simplex because of the negative results of intracerebral inoculations of emulsified brain into rabbits.*

In view of the present state of knowledge as to the relation which exists between inclusion bodies and filterable viruses, it is suggested that in this particular case a filterable virus may have been associated with the lesions. That this is a case of encephalitis there can be no doubt. From the history, physical examination and laboratory findings, it fits quite well into the amyostaticakinetic form of encephalitis lethargica, the terminal attack being an acute exacerbation of a preëxistent infection of sixteen months duration.

A second case has recently come to us which presented a typical clinical picture of an acute lethargic encephalitis of only forty-eight hours duration. Postmortem examination of the brain revealed gross congestion, edema, microscopic hemorrhages and capillary thrombosis, unassociated with perivascular or epivascular lymphocytic infiltration. Careful examination of the nerve cells revealed extensive necrosis and some pseudoneuronophagia. No inclusions were observed.

Another case examined at autopsy was a typical clinical encephalitis lethargica coming on after extensive burns over the body, several weeks elapsing between the burns and the development of the symptoms referable to the central nervous system. Grossly, little could be demonstrated other than congestion of the basal ganglia. Microscopically, there were numerous lymphocytes within the arterial walls, extensive necrosis, a diffuse mononuclear exudate, and

* The material used for animal inoculation consisted of small fragments of basal ganglia which had been preserved in 50 per cent glycerine-saline solution and kept at freezing temperature for 1 month and 16 days. This material was washed, ground and emulsified in saline and inoculated intracerebrally into 2 monkeys (*M. rhesus*), 9 mice, 1 guinea pig, and 17 adult rabbits. One rabbit was inoculated in the testis.

little hemorrhage. The lesions were almost entirely confined to the basal ganglia. Here again no inclusions were demonstrated, and animal inoculations were negative.

These three cases may well be included in the group of encephalitis lethargica. Each is distinctly different from the other clinically, as well as pathologically. In fact, they present only a few points of similarity. In only one of them have inclusion bodies been demonstrated. In this particular case there is strong morphological evidence which points to a filterable virus as the etiological agent. Careful study of the other two cases revealed no inclusions, and it is difficult to believe that other investigators could have overlooked them in cases previously reported. It must be concluded that the case here described differs from others heretofore recorded in this essential feature.

If, then, in this small series there is evidence pointing to at least one cytological difference between cases which are called at present epidemic or lethargic encephalitis, it may be concluded that encephalitis lethargica is probably not a clinical or pathological entity, but that it represents an ill-defined group characterized by varied clinical and pathological findings and probably caused by various agents.

Experimentally, considerable work was done with material from all three of our cases, using fresh and glycerinated tissue from the basal ganglia, cortex and medulla as inocula. Material from the three cases was washed, ground, emulsified in saline and inoculated into a total of four baby rabbits, thirty-one adult rabbits, baby mice, adult mice, two puppies, three monkeys and several guinea pigs and cats. In most instances the emulsified brain was inoculated intracerebrally, although several attempts were made to infect the rabbit's cornea. In rabbits, monkeys and guinea pigs, 0.5 to 1 cc. of a thick emulsion was inoculated. Following Flexner's work on poliomyelitis attempts were made to infect animals by following the intracerebral inoculation with intraperitoneal injections of emulsified brain. The animals were watched for varying periods. Examination of their brains revealed not the slightest suggestion of any inflammatory process comparable to that seen in encephalitis.

Many of the animals survived without manifesting any clinical changes. These were observed over periods varying from three to six months. Careful temperature charts were kept on all animals.

In none of these animals was there any significant fever. Microscopic examination of the brains of animals which survived several weeks and were sacrificed showed no significant changes.

In another case, clinically diagnosed encephalitis lethargica (brother to Case 2), spinal fluid was inoculated intracerebrally into rabbits in 0.5 and 1 cc. quantities with negative results. This child survived the acute attack. At the present time, six months after onset, the child shows a reversal of the sleep cycle and some salivation, which is interpreted by the clinicians as evidence of encephalitic sequelae.

SUMMARY AND CONCLUSIONS

1. A case of lethargic encephalitis is reported in which intranuclear and intracytoplasmic "inclusions" occur.
2. It is suggested, on the basis of the presence of cellular inclusions, that this case of encephalitis may have been due to a cytotropic virus.
3. Two other cases of encephalitis are mentioned in which no inclusions were found.
4. It is further judged from an etiological standpoint that lethargic encephalitis may not be a distinct entity.
5. Animal inoculations with material from each case induced no demonstrable infection.

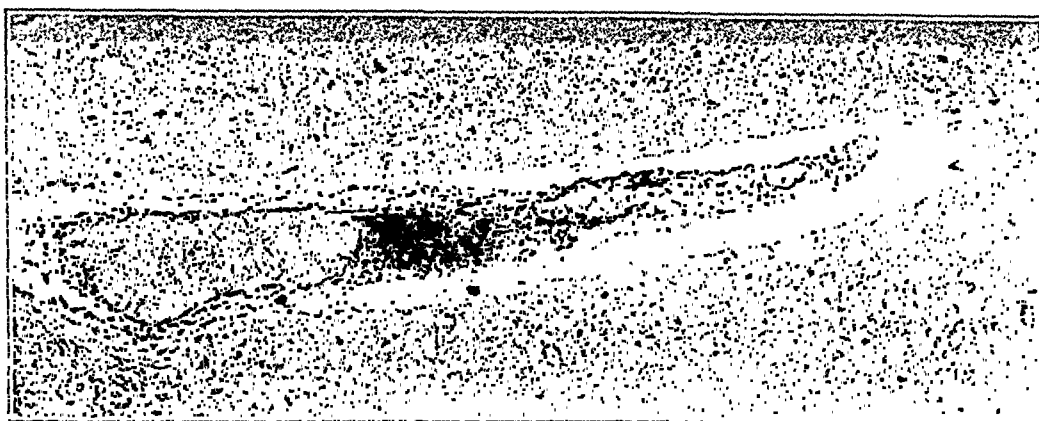
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DESCRIPTION OF PLATES

PLATE 3

- FIG. 1. Longitudinal section through small venule in the lenticular nucleus. The lymphocytes are seen in the adventitial coat. Hematoxylin and eosin stain. $\times 100$.
- FIG. 2. Large ganglion cell from left motor cortex showing several dense cytoplasmic inclusions surrounded by halos, and a single, poorly defined intranuclear inclusion. Phagocytic cells are beginning to accumulate around it. Eosin-methylene blue stain. $\times 1100$.
- FIG. 3. Pseudoneuronophagia of a ganglion cell from the caudate nucleus. The cell contains a definite intranuclear inclusion and a single pale, rounded cytoplasmic inclusion near the upper pole of the nucleus. Eosin-methylene blue. $\times 1100$.
- FIG. 4. Ganglion cell from left motor cortex showing the characteristic large granular inclusions and three poorly defined, homogeneous, cytoplasmic inclusions. This cell shows the characteristic condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. $\times 2500$.
- FIG. 5. Necrotic ganglion cell from pons surrounded by phagocytic cells. Eosin-methylene blue. $\times 1100$.
- FIG. 6. Ganglion cell from left motor cortex showing a granular intranuclear inclusion and a large homogeneous cytoplasmic inclusion. Eosin-methylene blue. $\times 2500$.
- FIG. 7. Two swollen neuroglial cells from caudate nucleus showing small intranuclear inclusion. Eosin-methylene blue. $\times 1100$.



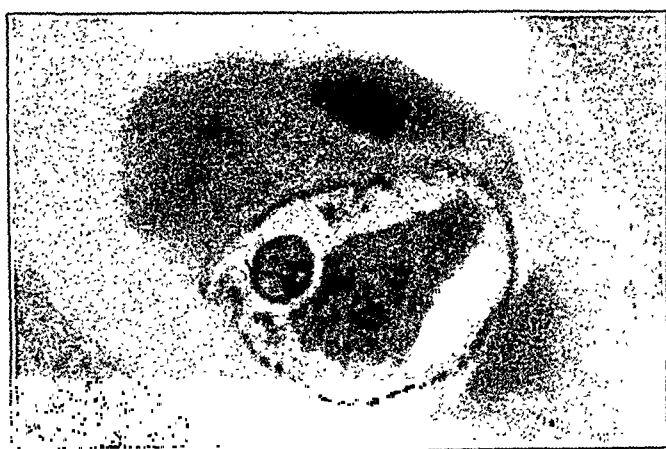
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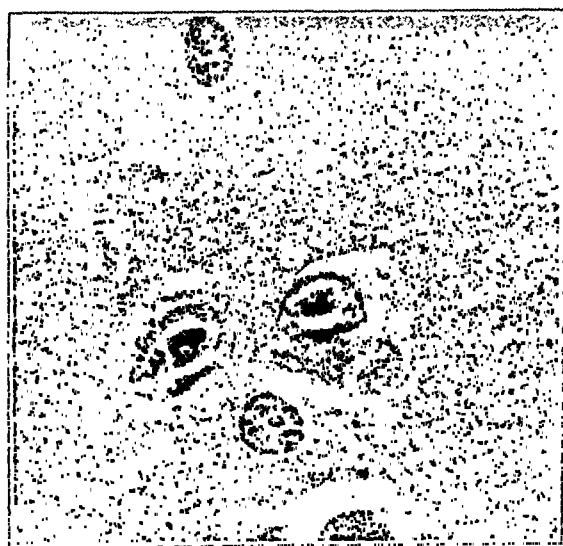
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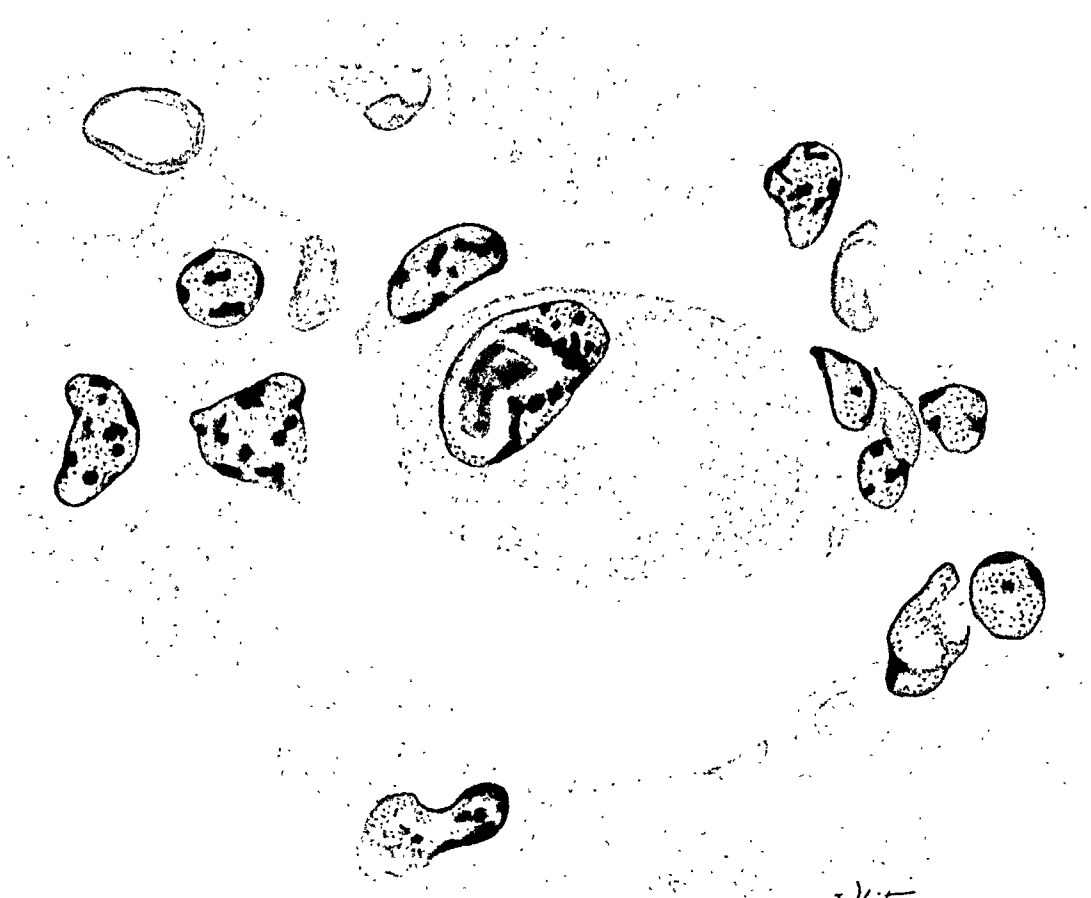
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PLATE 4

- FIG. 8. Ganglion cell from caudate nucleus showing an eosinophilic, granular, intranuclear inclusion and a poorly defined, round, homogeneous cytoplasmic inclusion. Surrounding the cell are many phagocytes. Eosin-methylene blue. $\times 2500$. (See Fig. 3 for photomicrograph of this cell.)
- FIG. 9. Ganglion cell from left motor cortex showing large, granular, eosinophilic intranuclear inclusion and three indefinite homogeneous cytoplasmic inclusions. The chromatic material is collected along the nuclear membrane. Eosin-methylene blue. $\times 2500$. (See Fig. 4 for photomicrograph of this cell.)
- FIG. 10. Ganglion cell from left motor cortex showing characteristic intranuclear inclusion. Eosin-methylene blue. $\times 2500$.
- FIG. 11. Ganglion cell from left motor cortex showing several reddish orange, homogeneous, cytoplasmic inclusion bodies which are surrounded by halos. There is also a large granular intranuclear inclusion. Eosin-methylene blue. $\times 2500$. (See Fig. 2 for photomicrograph of a similar cell.)
- FIG. 12. Ganglion cell from left motor cortex showing a single intranuclear inclusion. This cell also illustrates the condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. $\times 2500$.
- FIG. 13. Ganglion cell from caudate nucleus showing a typical intranuclear inclusion. Eosin-methylene blue. $\times 2500$.
- FIG. 14. Ganglion cell from caudate nucleus showing an intranuclear inclusion with some condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. $\times 2500$.



Anna White

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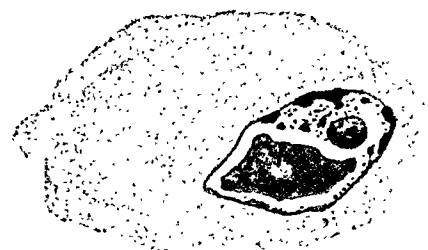
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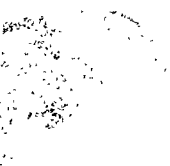


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Cellular Inclusions in Lethargic Encephalitis



TUBERCULOUS VEGETATIONS OF THE TRUNK OF THE PULMONARY ARTERY *

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Although nearly fifty cases of tuberculous involvement of the aorta have been reported, tuberculosis of the main stem of the pulmonary artery is rare. Only two cases are described in the literature and these deal with miliary lesions. The first case, reported by Weigert¹ in 1886, represents the extension of a tuberculous lymphadenitis into and through the vessel wall with production of miliary intimal tubercles. Thorel² mentioned a second case in which there were very minute miliary intimal tubercles associated with miliary tuberculosis of the main stem of the pulmonary artery. In view of the rarity of the condition the following case is reported.

REPORT OF CASE

Clinical History: L. B., a 65 year old negress, was admitted to the Medical Service of the Cleveland City Hospital complaining of a sore throat. The history of her illness indicated pulmonary disease of at least three weeks duration. The physical examination disclosed a negress of 65 years who appeared severely ill, and who showed evidence of marked loss of weight. The respirations were very rapid and shallow. Over the upper half of the right chest anteriorly, dullness and many fine râles were elicited. The heart sounds were distant and scarcely audible. The rate was rapid. The liver was moderately tender to palpation. The ankles and feet were edematous. The patient died on the day following admission.

POSTMORTEM EXAMINATION

At autopsy, two hours after death, there was observed a generalized miliary tuberculosis. The lungs, in addition, showed tuberculous pneumonia. There was an adherent pericardium. The heart with pericardium weighed 450 gm. Aside from a slight dilatation of the chambers, the heart was normal. At the bifurcation of the pulmonary artery there was a blue-gray, oval, intimal plaque 0.9 cm.

* Received for publication June 10, 1932.

by 1.5 cm., elevated about 1.5 mm. above the surface. From the middle of this plaque hung a soft, pink-gray polyp 1.5 cm. long and 0.6 cm. thick (Fig. 1). This polyp broke on handling, allowing a thick, pinkish yellow, turbid fluid to escape. A smear of this material showed dense masses of acid-fast bacilli with a little cellular débris. The surface of this polyp was granular and definitely nodular. Below this vegetation and slightly to the left, there was another pedunculated polyp 1 cm. long and extending upward from a point immediately to the left of the commissure joining the right anterior and posterior cusps. The stalk was cylindrical, 3 mm. in diameter and 5 mm. in length. The distal part was ovoid in shape, 5 mm. in diameter and 5 mm. long, and finely granular on the surface. It was gray in color and quite firm, although friable. At the base and to the left of this second vegetation there was a third polyp about 5 mm. in length with a granular surface and bifid upper pole. This polyp had the same color and consistence as the second. The valve leaflets and commissures were normal.

There was a marked tuberculous mediastinitis. Attached to the posterior aspect of the pulmonary artery, near its bifurcation, there were caseous lymph nodes which were also adherent to the tissues at the bifurcation of the trachea. There were numerous other caseous lymph nodes in the vicinity which were matted together by fibrous tissue.

MICROSCOPIC EXAMINATION

Microscopically the sections show tuberculous granulation tissue between the pulmonary artery and the aorta. While the latter is not affected by the disease, the pulmonary artery throughout its adventitia is severely involved by the tuberculous inflammation. Large areas of caseation necrosis with the usual lymphocytic and endothelial cells are seen. At various levels of the pulmonary artery the media is invaded and interrupted by the tuberculous granulation tissue (Fig. 2). In these situations direct continuity exists between the tuberculous tissue of the adventitia and that of the intima. The intimal disease is characterized by the presence of tuberculous granulation tissue, areas of caseous necrosis, and by medium-sized blood vessels whose walls are markedly thickened. The thickening is eccentric, encroaches upon the lumen and consists of disoriented, loosely arranged hyaline fibrils in which numerous, poorly staining,

large mononucleated cells are enmeshed (Fig. 3). There is also a marked hyaline swelling of the internal elastic lamina of the pulmonary artery to about twice its normal thickness (Fig. 4).

Further continuity exists between the tuberculous tissue of the intima and similar tissue composing the base of the polyp situated at the commissural level. At higher levels this polyp consists of a center of loosely arranged, acellular, hyalinized, fibrinous material and a periphery of more compact hyaline material in which a few lymphocytes are present. A section of the large polyp exhibits a thin marginal ring of compact hyaline material containing an occasional lymphocyte. Dense masses of acid-fast bacilli are present in the center of the ring. One section through the base of the smallest vegetation shows tissue similar to that of the adjacent polyp with which it is continuous. The exact relation of the smallest polyp to the tissues composing the wall of the pulmonary artery was not established.

The continuity of the caseous tuberculous lymphadenitis with the extensive adventitial involvement of the pulmonary artery, and the further continuity, through the interruptions in the media, with the intimal tuberculosis, indicate that the tuberculous arteritis probably began in the adventitia and that the intimal lesions represent extension by contiguity. It is possible that the miliary dissemination had its origin in the tuberculous polypi.

DISCUSSION

In Weigert's¹ case of vascular tuberculosis the disease began as a perivascular lesion which involved the intima only secondarily. Benda,³ however, reported cases of intimal tuberculosis (not involving the pulmonary artery) in which the disease was confined to the intima. This, he assumed, represented the lesion caused by implantation of tubercle bacilli upon the intact or diseased intimal surface. He claimed that this endangitis tuberculosa represented the more common form of vascular tuberculosis. Subsequent writers and more modern textbooks of pathology are agreed that the periangitic form, first described by Weigert, is by far more common than the endangitic form. Jores⁴ further emphasized the fact that tuberculous vascular involvement is more common with veins than with arteries.

The appearance of the intimal tubercle varies from a small,

slightly elevated nodule, miliary in size, to soft, pedunculated polypi 1 cm. in length, and larger. Thus Benda described a "very large" polyp of the main branch of the pulmonary vein, which hung almost into the left ventricle. The polypi are usually yellow or yellow-gray in color. The external surface has been described as smooth and also as nodular. The consistence is usually quite soft, although an occasional polyp of firm consistence is seen.

The contents of the soft polypi consist of caseous, pus-like material in which a varying number of acid-fast bacilli are found. Usually the bacillary content is very high. Benda called attention to the fact that these polypi are often ulcerated, even though the surface appears smooth and uninterrupted. A layer of hyalinized fibrin may cover the point of rupture and give it the appearance of a smooth surface. Such a lesion may then be the source of a disseminated miliary tuberculosis.

Although no polypi of the pulmonary artery have been described, the description of polypi found in the aorta and pulmonary veins as given above adequately covers the vegetations found in the pulmonary artery of this case.

SUMMARY

1. A third case of tuberculous involvement of the main stem of the pulmonary artery has been described.

2. The tuberculous involvement in this case is the result of an extension of a tuberculous lymphadenitis from adjacent lymph nodes to the adventitia and thence to the intima of the pulmonary artery.

I am indebted to Prof. Howard T. Karsner for helpful criticism and suggestions in the preparation of this paper and to Dr. Alan R. Moritz for the photomicrographs.

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DESCRIPTION OF PLATES

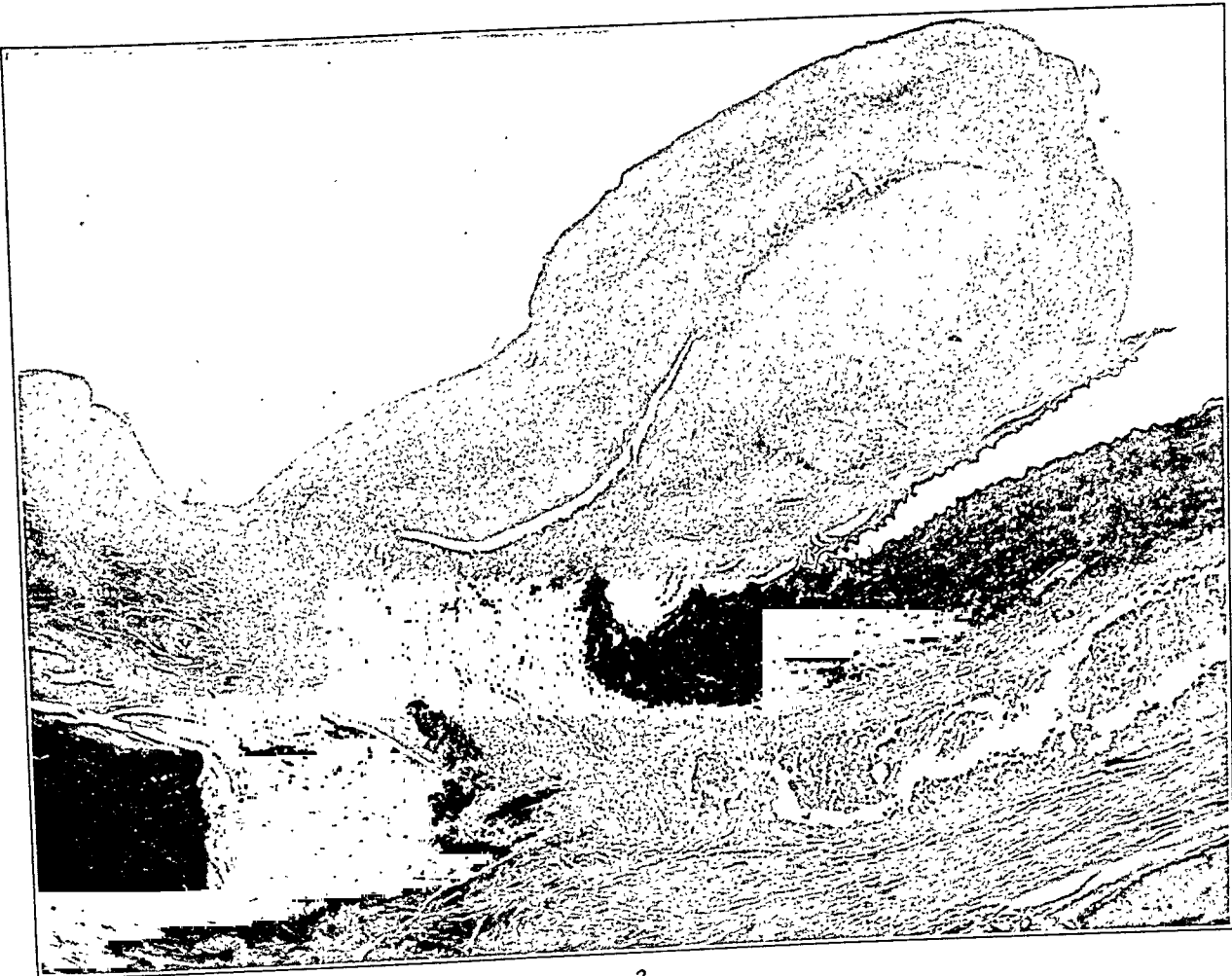
PLATE 5

FIG. 1. Drawing of heart and pulmonary artery showing three polypoid tuberculous vegetations attached to the wall of the pulmonary artery.

FIG. 2. Wall of the pulmonary artery at the commissural level with attached vegetation, illustrating the continuity of the tuberculous granulation tissue in the adventitia with that of the vegetation through an interruption in the media. Weigert's elastic tissue stain. $\times 20$.



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Tuberculous Vegetations of Pulmonary Artery

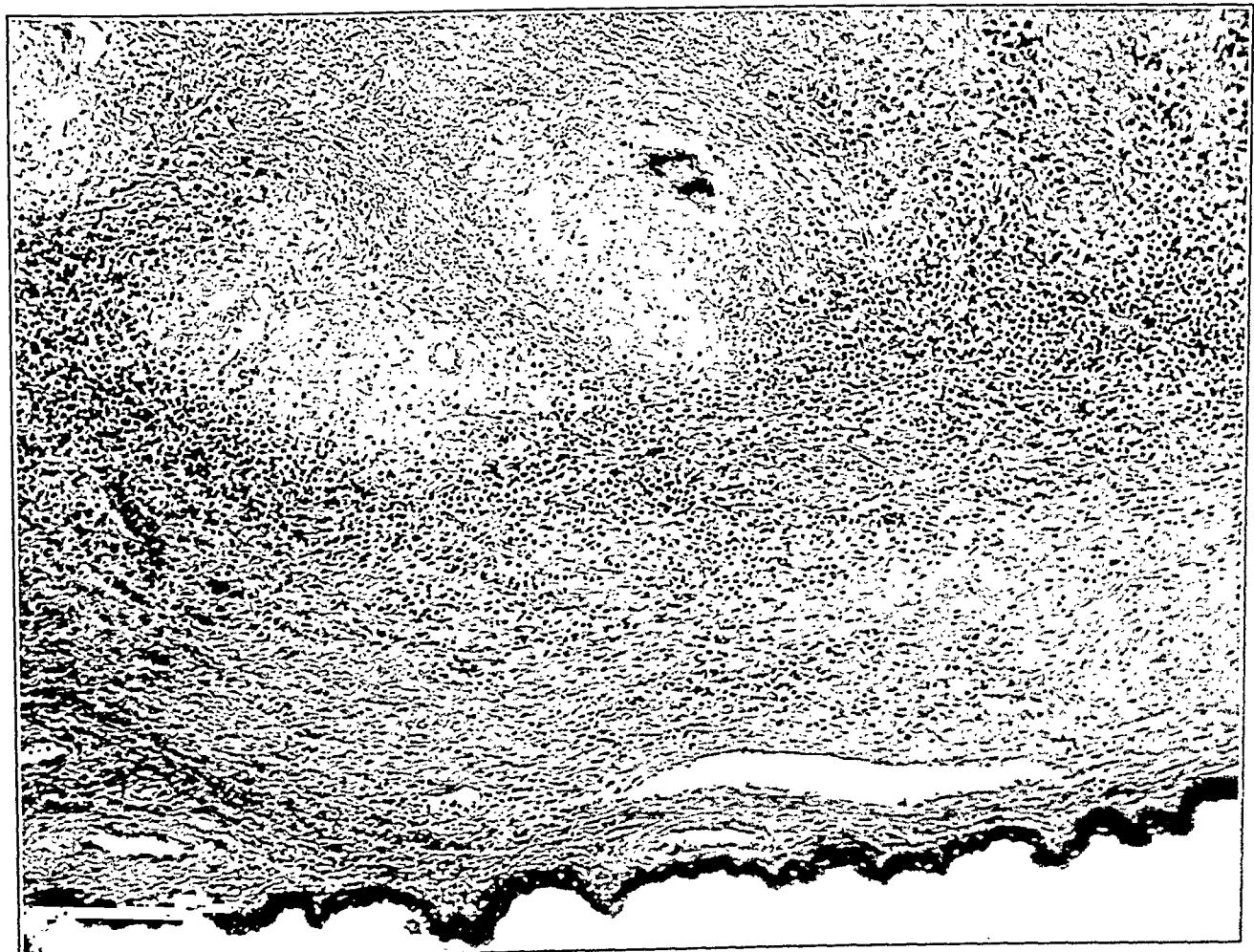
PLATE 6

FIG. 3. Intima and a small portion of the media, showing a blood vessel in the intima surrounded by tuberculous granulation tissue. Hematoxylin and eosin stain. $\times 110$.

FIG. 4. Tuberculous granulation tissue in the intima and hyaline swelling of the internal elastic lamina. Weigert's elastic tissue stain. $\times 134$.



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4



MICROINCINERATION STUDIES OF HUMAN CORONARY ARTERIES *

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The method of microincineration of microscopic specimens of tissues devised by Liesegang was revived and improved by Policard, Tschopp, and by Scott. It has been employed for numerous studies in the microscopic anatomy of the distribution of salts in various parts of the body. Pathology has profited little as yet except for a few studies, notably those of Schultz-Brauns. Zinkant studied the arteries of the uterus by this method and showed what he believed to be the beginning of arteriosclerotic changes comparatively early in life. There is, however, reasonable doubt that the deposit of calcium within the arterial walls, as revealed by the method of microincineration, necessarily represents a pathological change. The present study deals with the ash left by the coronary arteries of the heart following microincineration, and considers the matter in reference to age periods.

METHOD AND MATERIALS

In general, the method as described by Policard was employed. The material was from 62 human hearts at different ages. From each heart four blocks were made. One block was in vertical and another in tangential section from the wall of the left ventricle midway between apex and base near the septum; one vertical section from the anterior border of the right ventricle near the septum and a tangential section just anterior to the margo acutus were also made. These blocks, from 1 to 2 cm. square and not more than 5 mm. thick, were taken before the heart was washed and placed immediately in a solution of 9 parts absolute alcohol to 1 part neutral formalin (40 per cent formaldehyde, C.P.). After not less than 24 hours' fixation the material was placed in absolute alcohol and, after about 6 transfers at 2 hour intervals, embedded in paraffin by the usual technique

* Received for publication June 17, 1932.

and cut in sections 6 microns thick. Five sections were made from each block. Sections 2 and 4 were stained with hematoxylin and eosin or orcein and hematoxylin. For sections 1, 3 and 5 slides were freed of grease with the utmost care, thoroughly cleaned and placed upon a warm stage at about 45° C. A few drops of alcohol were placed upon each slide and the section placed upon this alcohol. The warmth permitted the sections to flatten out smoothly and the material was then dried in air. In our experience water has proved to be quite as satisfactory as alcohol and does not appear to dissolve any of the salts out of the tissue. Incineration was carried out in a small electric oven so adjusted by rheostat that it maintained a temperature of 650° C. Incineration was continued until all charred material had disappeared and the sections lay as a white tissue upon the slide. The section was covered with a coverglass and sealed with paraffin. The examinations were with dark-field illumination, as suggested by Scott. The materials were grouped according to decades and the accompanying tables give an outline of the findings.

RESULTS

First Decade: (Table I) This group included 10 cases ranging in age from newborn to 8 years. The gross findings as regards coronary arteries were negative throughout and this is true as regards microscopic findings, except in Case 10 in which typical rheumatic infiltrations were found in the myocardium and around the coronary vessels. In this decade the incinerated preparations appeared to show a smaller number of blood vessels than was observed in the hematoxylin and eosin preparations, but this dissimilarity was found to be due to the fact that no observable ash was produced by the very small vessels. In the position of the intermediate arteries there was found a ring of ash which in the younger children appeared as a row of fine beads, but in the older children as a continuous line. At times minute nodules appeared to project into the lumen of the vessel. This ring of ash is interpreted as representing the intima, more especially the endothelial cells of that coat. With the advance of age the line, which became continuous, also became increased in density. In the younger children the media and adventitia were not recognized by the ash, but in the older children a few small dots of ash were found in both coats. The exception to this was in that of Case

10 where the ash in adventitia and perivascular connective tissue was distinctly increased, apparently because of the cellular content in this region.

Second Decade: (Table II) Of the 4 cases in this group from 15 to 20 years of age, 2 showed slight beginning atherosclerosis of the coronaries grossly, and the other 2 showed no change. The sclerosis was almost completely confined to the larger branches, but in Case 4 of this group the smaller vessels in both ventricles showed slight intimal fibrosis. The principal difference discovered between this group and the patients in the first decade was the increase in amount of ash found in the intima. The adventitia and media left about the same amount of ash as in the older children of the preceding decade. In Case 4 mentioned above, a patient 20 years of age, the intima was distinctly richer in ash, and the media showed slightly more ash than in the other cases. A distinction between the ash of the three layers of the arterial wall was not easily possible.

Third Decade: (Table III) This group included 9 cases from 25 to 30 years of age. Grossly, all showed slight atherosclerotic change in the larger divisions, with the exception of Case 8 in which the artery appeared to be normal. With the hematoxylin and eosin preparations it was found that in 3 of these cases the sclerosis of the smaller arteries was confined entirely to the left ventricle, whereas in the other it was found in both ventricles. The intima in these cases showed moderate thickening, together with splitting and apparent multiplication of the internal elastic lamina. In 4 instances there was no manifestation of arteriosclerosis in the smaller vessels microscopically. In this age group it became possible to distinguish the ash of intima, media and adventitia. Generally speaking, the ash of the intima was greater in amount than in the preceding group and was especially prominent in the sclerotic areas. In 1 case without sclerosis the ash of the intima was definitely increased, as compared to earlier age groups, but the elastica stain preparation showed very clearly an increase in the amount of elastic tissue in the intimal coat. In certain of the arteries of Case 7, a leukemic patient, the ash appeared as tiny spots in the adventitia, corresponding to areas of leukemic infiltration. There were three female patients in this group and the amount of ash in the intima was definitely less than was true of the males.

TABLE I
First Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	Newborn	M	Hemorrhage of meninges and lungs	Negative	Arteries delicate. Ash even of larger intramuscular branches varies a little as a discontinuous ring, in media and adventitia as tiny spots
2	5 mo.	M	Bronchopneumonia	Negative	Arteries negative. Ash content as in preceding case
3	8 mo.	M	Spina bifida, acute purulent spinal meningitis	Negative	Small artery well contracted. Wall appears to be thickened. Intima of large artery gives a little more ash, less from media and adventitia
4	9 mo.	F	Influenza meningitis	Negative	Ash of intimal layer as a sharp ring with a little irregular outline
5	11 mo.	M	Embryonic tumor of left kidney	Negative	Ash of intimal layer as a sharp ring with a little irregular outline
6	3 yrs.	M	Acute purulent meningitis	Negative	No pathological changes. Ash in three layers of larger artery slightly increased
7	3½ yrs.	M	Enteritis follicularis	Negative	No pathological changes. Ash in intima of large arteries as a ring with slight irregularity in outline Very little ash in media and adventitia

8	5 yrs.	M	Bronchopneumonia	Negative	No pathological changes. Ash in intima of large arteries as a ring with slight irregularity in outline. Very little ash in media and adventitia
9	6 yrs.	M	Acute purulent meningitis	Negative	No pathological changes. Ash in intima of large arteries as a ring with slight irregularity in outline. Very little ash in media and adventitia
10	8 yrs.	F	Chronic rheumatic endocarditis	Negative	In adventitia and perivascular region of intramuscular arteries often infiltration of large and small mononuclear cells is seen. Ash in media and adventitia of some large arteries increased as tiny spots. Ash in small artery as distinct ring

TABLE II
Second Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	years 15	F	Poliomyelitis, broncho-pneumonia	Negative	Internal elastic lamella of intima very well developed, especially on left side. All intramural arteries give distinct ash ring in intima
2	17	M	Chronic ependymitis, hydrocephalus	A few small atheromatous patches in the left anterior descending branch. Right coronary artery negative	Slight proliferation of elastic fibers in intima of a few intramural arteries of left ventricle. Ash picture about the same as in preceding case
3	18	M	Chronic tuberculosis of lungs and spine	Negative	Intima of arteries no change, gives quite distinct ash ring
4	20	M	Generalized tuberculosis	In intima of beginning portion of left anterior descending branch a few yellowish streaks. Right coronary artery negative	Thickening and fraying out of internal elastic lamella of arteries of some trabecular muscles of both ventricles. Ash of large arteries somewhat increased in every layer, especially in intima of diseased arteries

TABLE III
Third Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	years 25	M	Cerebral hemorrhage, bronchopneumonia, cardiac hypertrophy	Atheromatous patch in the intima of the left anterior descending branch. Right coronary artery negative	Slight patchy thickening of intima and internal elastic lamella of a few intramuscular arteries, giving increased ash of small arteries, very distinct in intima
2	26	M	Chronic ulcerative pulmonary tuberculosis	A number of atheromatous patches in left anterior descending branch. A few minute yellow spots in right coronary artery	Slight thickening and fraying out of internal elastic lamella of a few middle-sized arteries. Ash in intima of arteries quite distinct
3	27	M	Lobar pneumonia	In left circumflex a yellow spot. Right coronary artery negative	Patchy thickening of a large artery of left ventricle. Increase of adventitial tissue. Ash increased in all layers of large artery. Ash in intima of arterioles very distinct
4	28	M	Bilateral lobar pneumonia	Left anterior descending artery shows slight thickening of the intima around an orifice of its branch. Right coronary artery negative	Internal elastic lamella of arteries very well developed. Ash found chiefly in intima. Little ash from media and adventitia
5	29	M	Acute mastoiditis, purulent cerebrospinal meningitis	Left coronary artery shows slight patchy thickening of the intima. Right similar to left	Patchy thickening of intima of a few large and small arteries with increased elastic fibers. Ash in intima as a rather thick, irregular ring. Ash in media of large artery also increased

TABLE III (Continued)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
6	29 years	M	Ulcerative pulmonary tuberculosis	An atheromatous patch in the intima of the left anterior descending artery. Right the same as left	Changes similar to those of Case 4
7	29	F	Chronic myeloid leukemia	Left coronary artery shows patchy thickening of intima in some places. Right coronary artery shows similar changes, but to a lesser degree	Wall of arteries thin and delicate. Ash of intima little in amount, but in media and adventitia increased as numerous tiny spots
8	29	F	Chronic pulmonary tuberculosis	Both coronary arteries negative	Wall of arteries thin and delicate. Ash of intima slight in amount
9	30	F	Purulent osteomyelitis, streptococcus sepsis	In both coronary arteries a few atheromatous patches	Similar to Case 8

TABLE IV
Fourth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	years 34	M	Bronchopneumonia	Left coronary artery shows a few atheromatous patches found in anterior descending branch. Right coronary artery shows numerous fine yellow spots	Arteries show no definite sclerotic changes in hematoxylin-eosin and elastic preparations. Ash in wall of medium-sized and smaller arteries as more or less irregular, quite clearing. Ash in media of large artery very slight
2	35	F	Infectious encephalomyelitis	Slight thickening of intima around some orifices in left anterior descending artery. Right coronary artery the same	Intima of a few large arteries of left ventricle slightly thickened. Ash picture similar to that of preceding case
3	36	M	Generalized tuberculosis	In both coronary arteries a few atheromatous patches	No arteriosclerotic changes. Ash in small arteries as distinct ring
4	37	M	Gangrene of lower extremities	A few atheromatous patches in left anterior descending artery. Many in the right coronary artery	A few middle-sized and small arteries of left ventricle showing thickening of intima and proliferation of elastic fibers, giving a thick ash ring or with frayed-out processes, or ring with double contour. Changes of artery of right ventricle similar
5	38	M	Bronchopneumonia	A few atheromatous patches in beginning portion of left anterior descending branches. Right coronary artery negative	Many arteries show thickening of intima, especially large arteries. Proliferation of elastic fibers in intima. Ash considerably increased in intima, also in media and adventitia
6	39	M	Generalized tuberculosis	Many fine yellow spots in intima of anterior descending branch of left coronary artery. Right coronary artery similar to a lesser degree	Similar to Case 3

Fourth Decade: (Table IV) In this group there were 6 cases from 34 to 39 years of age. In all cases the gross examination showed varying degrees of atherosclerosis in the larger branches of the coronary arteries. The ash of the intima as compared with the preceding age group was not only greater in amount but showed increasing density. In some of the cases the delimitation between intima and media was obscured because of what appeared to be a "fraying out" of the ash into the intima at points where the ash in the two coats could not be distinguished. Generally speaking, the ash of media and adventitia was clearly separable and appeared as a delicate network in both coats, increased in amount over the age group preceding. In Case 5 the amount of ash in all three coats was definitely greater than in the other members of this group.

Fifth Decade: (Table V) This group was made up of 14 cases ranging in age from 42 to 50 years. The gross examination showed definite intimal atherosclerosis in 12 of these 14 cases, and in Case 5 this was particularly prominent. Microscopically, with the hematoxylin and eosin stains, sclerotic changes were observed in smaller vessels of all these cases. These changes were, as a rule, distinctly more marked in the left ventricle than in the right. The ash from the arteries of all these cases was increased from all three coats as compared with the preceding age period, except in Case 9. In this instance, where there was no arteriosclerosis grossly and only slight sclerosis microscopically, the ash of the intima formed a thin, delicate ring and the ash of adventitia and media was very slight in amount. In those small divisions where sclerosis was prominent, the ash of intima showed a corresponding irregularity in relation to the lumen of the vessel.

Sixth Decade: (Table VI) Of the 10 cases of this group, ranging in age from 52 to 60 years, all showed noteworthy gross atherosclerosis of the coronaries and in 5 of the cases this was accompanied by small areas of calcification. The hematoxylin and eosin preparations showed varying degrees of intimal sclerosis in the smaller branches, but these changes were not parallel to those observed in the large divisions. In this group and in the preceding group sclerotic changes in the smaller vessels were observed, especially in the branches to the trabeculae carnae. In general the ash of the intima was considerably increased in amount over the preceding age group, corresponding closely to the sclerosis present in the vessels studied. In some of

the small arteries the amount of ash was so great that the lumen appeared to be almost occluded. The media and adventitia both showed a large quantity of ash. In many instances the perivascular connective tissue showed a delicate network of ash corresponding in general to the distribution of the loosely arranged collagenous fibers. With the dark-field illumination the ash of the perivascular tissue was of a pale blue color, as compared with the white of the coat of the vessels. In 1 case, illustrated in Figure 7, the ash of intima was in the form of delicate, radiating bands corresponding to the arrangement of elastica in that coat.

Seventh Decade: (Table VII) The 5 cases in this group were 61 to 70 years of age. The findings were so closely similar to those in the sixth decade that no separate description seems justified. The changes were essentially the same in all respects, except that an impression was gained that the amount of ash was somewhat increased and slightly more irregularly distributed.

Eighth Decade: (Table VIII) Of the 5 cases included in this group, 71 to 79 years of age, all showed definite coronary sclerosis grossly. Again the changes found in the smaller divisions were not parallel to those found in the larger arteries. In the hematoxylin and eosin preparations the intima showed nodular thickening in many instances and the amount of elastica in the patches was increased, as compared with the rest of the intima. The incinerated specimens showed a definite increase of the ash in the walls of almost all the arteries and this was especially marked in the smaller vessels. In these the ash of the intima was characterized by irregularities, apparently due to the patchy thickening. The ash of media, adventitia and perivascular connective tissue was markedly increased, quite regardless of the degree of sclerosis.

DISCUSSION

The incinerated preparations show ash from the coronary arteries in all the ages included in this study. In young subjects the ash of small branches may be so small in amount that it does not form a continuous ring. In the first three decades there is a progressive increase in the amount of ash, but this does not in all instances parallel the advance of age. Exceptions are especially noteworthy in the females of the third decade. Limitations of time and material made it impossible to distinguish as to sex differences, but it is assumed

TABLE V
Fifth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	3 years 42	M	Cellulitis of scrotum and pelvic tissue	Slight thickening of intima around orifices of branches of left anterior descending artery. The same in the right coronary artery	Some large and small arteries with patchy thickening of intima and increase of elastic fibers of internal elastic lamella. In hematoxylin and elastic stains changes of artery of right ventricle definitely less in degree. A great deal of ash found in intimal layer. Little in media and adventitia of large artery
2	44	M	Generalized arteriosclerosis, luetic aortitis	Slight thickening of intima around orifices of branches of left anterior descending artery. The same in the right coronary artery	Similar to Case 1
3	44	M	Generalized tuberculosis	A few atheromatous patches in intima of left anterior descending branch. Right coronary artery shows only one yellowish patch	Slight patchy thickening of intima of large artery. Ash in large and small arteries little in amount
4	45	M	Acute toxic hepatitis	Left coronary artery slightly stiff, showing some patchy thickening of its intima	Slight arteriosclerotic changes of large arteries. Considerable amount of ash, especially in intima of arteries
5	46	F	Hypertension, syphilitic aortitis	Marked arteriosclerosis with stiffness and atheromatous changes of coronary arteries, more on left side	Considerable thickening of intima with increase of elastic fibers in all arteries, especially those of left ventricle. Ash much increased in all layers of artery, especially the intima. Little ash also increased in perivascular fibrous tissue

6	46	M	Generalized tuberculosis	In beginning portion of left anterior descending artery numerous atheromatous patches. Right coronary artery free	Thickening of intima and increase of elastic fibers of internal elastic lamella of most arteries, especially small ones. Artery of left ventricle shows more marked changes than that of right. Ash increased in all layers
7	46	M	Lobar pneumonia	A few atheromatous patches in left anterior descending branch. Right coronary artery same changes	Changes similar to those of Case 6, but to a higher degree
8	46	F	Bronchopneumonia, generalized arteriosclerosis	In beginning portion of left anterior descending branch a few atheromatous patches. Right coronary artery shows numerous confluent yellow patches. Same thing in circumflex artery	Much thickening of intima of arteries, narrowing of their lumina. Thickening of intima chiefly due to increased fibrous tissue. Elastic fibers of internal elastic lamella slightly increased. Two kinds ash: faintly illuminated in fibrous part of intima and brightly illuminated in elastic part
9	47	M	Bronchopneumonia	Both coronary arteries negative	All thin-walled arteries giving ash in form of a delicate ring
10	47	F	Cancer of breast with extensive ulceration	Both coronary arteries negative	Similar findings as in Case 1
11	48	M	Bronchopneumonia	Slight thickening of intima around orifices of branches of left anterior descending branch. Right coronary artery same change	No arteriosclerotic changes found, but in intima a distinct ash-ring is observed
12	49	F		A few minute yellow spots in beginning portion of left anterior descending branch. Right coronary artery negative	Slight thickening of a few large and middle-sized arteries. Ash picture similar to that of Case 11

TABLE V (Continued)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
13	50 years	M	Chronic pulmonary tuberculosis; cardiac hypertrophy and dilatation	A few atheromatous patches in large branches on left side. Right coronary artery shows one atheromatous patch in its beginning portion	Elastic fibers of intima of some intramuscular arteries considerably increased. Ash of small arteries as a thick ring
14	50	M	Cerebral hemorrhage; arteriosclerosis of aorta; cardiac hypertrophy and dilatation	A few atheromatous patches in beginning portion of left anterior descending branch. Right coronary artery stiff with marked atheromatous changes	Small arteries, especially in the trabecular muscles of both ventricles, arteriosclerotic with increased elastic fibers in intima. Ash of such arteries as a thick ring irregular in outline. Large and middle-sized arteries changed in similar manner, but less marked

TABLE VI
Sixth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	years 52	M	Chronic pulmonary tuberculosis, cardiac hypertrophy and dilatation	A few atheromatous patches in left anterior descending branch. Right coronary artery the same	Large and middle-sized arteries slightly arteriosclerotic, but small ones show severe changes with obliteration of lumina by reproduction of elastic fibers in intima. Ash of such arteries greatly increased
2	52	F	Generalized arteriosclerosis, cardiac hypertrophy and dilatation	Lumina of large branches of left coronary artery much narrowed, with numerous calcified patches. Right coronary similar to left	Intramuscular arteries of deeper layer of left ventricle quite arteriosclerotic with increased ash amount. Arteries of superficial layers and of right ventricle slightly changed
3	54	F	Generalized arteriosclerosis, cardiac hypertrophy and dilatation	Large branches of left coronary artery almost diffusely occupied by atheromatous and calcified patches. Some hyaline plaques and atheromatous patches in right coronary artery	Considerable degree of arteriosclerosis of a number of intramuscular arteries. Ash considerable in amount in intima of such arteries
4	54	F	Cancer of breast	Left coronary artery, beginning portion of anterior descending branch shows an atheromatous patch. Right coronary artery negative	Pronounced arteriosclerosis almost entirely limited to arteries of trabecular muscles of left ventricle with increased ash in intima. Slight increase of ash in media of large and middle-sized arteries
5	55	M	Chronic pulmonary tuberculosis	A few small atheromatous patches in left anterior descending branch. Right coronary artery shows a large atheromatous patch	Similar changes as in preceding case

TABLE VI (Continued)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
6	years 55	M	Gangrenous cystitis, pyonephritis	Thickening of wall and narrowing of lumen of all coronary arteries. Intima with numerous atheromatous and calcified patches. Changes on left side more marked	Moderate degree of arteriosclerosis of intramural arteries of deeper layer of myocardium of left ventricle, less of right ventricle. Ash in intima increased, same in media and adventitia of larger arteries
7	58	M	Spinal cord tumor; generalized arteriosclerosis	Coronary arteries on <i>both sides</i> stiff and their lumina narrowed with many atheromatous calcified patches	Similar to preceding case
8	58	M	Cancer of esophagus	Numerous atheromatous and calcified patches in intima of beginning portion of left anterior ascending branch. Right coronary artery similar to left	Intramural arteries of deeper layer of myocardium of left ventricle greatly thickened. Elastic fibers much increased. Ash in all layers, especially in intima, increased
9	58	M	Generalized arteriosclerosis and lobar pneumonia	Marked atheromatous changes of intima of both coronary arteries	Marked arteriosclerotic changes of all arteries, especially small arteries. Ash increased in three layers
10	60	M	Syphilitic aortitis, generalized arteriosclerosis	Slight thickening of intima of left anterior descending artery around orifices of its branches. Right coronary artery negative	Moderate arteriosclerosis of middle and smaller sized arteries of left ventricle. Arteries of right ventricle negative except for a few small ones. Ash of intima of small arteries slight in media, and in adventitia of large and middle-sized arteries increased

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TABLE VII
Seventh Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	years 61	M	Arteriolar nephrosclerosis, cardiac hypertrophy and dilatation	Thickening and atheromatous changes of intima around orifices of branches of both coronary arteries	Considerable degree of arteriosclerosis of all intramural arteries. Thickening and reproduction of elastic fibers of intima of trabecular arteries in left ventricle marked. Ash in intima increased
2	62	M	Tabes dorsalis, syphilitic aortitis, bronchopneumonia	Left anterior posterior descending and circumflex artery shows many atheromatous and calcified patches in intima. Right coronary artery, similar changes, but limited to beginning portion	Fairly marked arteriosclerotic changes of all arteries of both ventricles. Ash findings similar to those of preceding case
3	65	M	Chronic pulmonary tuberculosis	Coronary arteries on both sides show some hyaline patches around orifices of their branches	Marked arteriosclerosis of small arteries of trabecular muscles of both ventricles. Ash increased in small arteries, also slight amount in media and adventitia of middle and large arteries

TABLE VII (Continued)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
4	67 years	M	General arteriosclerosis and thrombosis of both coronary arteries	Left coronary artery with marked atheromatous changes in all large branches. Calcified patches of anterior descending branch and old thrombus in its beginning portion. Right coronary artery shows similar changes and fresh thrombus in its beginning portion	Marked arteriosclerosis, chiefly of large and middle-sized arteries with increase of elastic fibers and connective tissue in intima. Changes of artery of left ventricle more marked. Increased elastic fibers in adventitia and perivascular region. Ash increased in amount, especially in intima
5	70	M	Cancer of bladder	Marked arteriosclerosis of coronary arteries on both sides with numerous calcified and atheromatous patches in intima, their intima being greatly narrowed. Occlusion of left anterior descending branch	Similar changes as in Case 4, but to a lesser extent

TABLE VIII
Eighth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	years 71	M	Coronary thrombosis, right	Stiffness and narrowing of all large coronary branches showing numerous atheromatous and calcified patches, especially in left anterior descending branch. Thrombosis of right coronary artery	Arteries generally thick-walled with increased amount of elastic fibers in intima, in certain places also in perivascular region. Ash increased considerably in intima
2	72	M	Carcinoma of gall bladder	Numerous atheromatous patches in left anterior descending branch. Right coronary artery negative	Marked thickening of intima of a few intramural arteries with much increase of elastic fibers in intima. Ash in intima and media rather increased
3	73	M	Generalized arteriosclerosis, encephalomalacia	Many atheromatous patches, some of them calcified, especially in left anterior descending branch. Right coronary artery much narrowed	Marked arteriosclerosis of small and middle-sized arteries. Internal elastic lamella greatly increased in thickness. Lumen much narrowed. Increased amount of ash in intima
4	75	M	Bronchopneumonia	Many atheromatous and calcified patches of left coronary artery. Right coronary artery similar, but to a lesser degree	Advanced arteriosclerosis of small arteries of both ventricles. Internal elastic lamella thickened, chiefly in patchy form and frayed out. Ash increased, especially in intima
5	79	M	Generalized arteriosclerosis, cardiac hypertrophy and dilatation	Numerous atheromatous and calcified patches in intima of large branches of left coronary artery. Wall stiff and lumen narrowed. Right coronary artery with many atheromatous patches	Most intramural arteries show thickening of internal elastic lamella, usually partially button-like, protruding into lumen. Changes on left side more prominent. Ash of intima increased chiefly in arteriosclerotic arteries

that generally the coronaries of the female probably show less ash than those of the male. The increase in amount of ash affects all the arteries studied. After the third decade, although there is a progressive increase in amount of ash, the progression lacks the regularity observed in the first three decades. The greatest amount of ash was observed not in the eighth decade, but in a 58 year old male patient. The variation between arteries of the same case in these later age periods is greater than in the earlier periods. In the sixth decade the greatest amount of ash was found in those arteries supplying the trabeculae carneae. In the seventh and eighth decades the increase in amount of ash affected all the arteries studied. Thus, there is considerable variation in the increase in ash in these last three decades. The confusion is produced principally because of the varying degrees of arteriosclerosis, which in this series of cases was found to be most marked in the sixth decade.

The factor of arteriosclerosis is of much less significance in the first three decades and it would appear that the increase in amount of ash in these periods is parallel to the rate of growth of the body. In Case 12 of the first decade the pathological changes noted are responsible for the variation in ash content. Zinkant in his study of uterine arteries found no ash in these vessels of most of his cases in the first decade. There was a gradual increase of ash during the second decade. This he regarded as a deposit of calcium and probably incorrectly, as indicated below, interpreted it as a pathological change resulting from the functional alterations in the organ, a precursor of arteriosclerosis. Schultz-Brauns found ash in the aorta of a newborn and of a young child, but did not discuss its significance.

The present study indicates that the greatest amount of ash is in that part of the artery rich in elastic fibers. Figure 10 shows the position of ash in the media of the aorta especially rich in elastica. When compared with the renal artery (Fig. 11) which is a muscular type of vessel, the latter shows the ash especially about the internal elastic lamina. In the coronary arteries the ash is noteworthy in the intima, but generally is in comparatively small amounts in media and adventitia. The increase observed in the coronary arteries during the first three decades corresponds to the normal growth of the arteries and the "aufsteigende Periode" of Jores. Thus, a certain amount of ash must be considered as normal and not of necessity an indication of sclerosis, except in so far as sclerosis may be looked

upon as a process of aging. In these earlier decades where sclerosis is not demonstrable, the ash occurs in fairly regular ring or oval forms, but when sclerosis becomes noteworthy the ash lacks uniformity in arrangement, is often eccentric in distribution and variable in quantity. It is probable that the same argument explains the small amount of ash in the coronaries of the female. Since the largest amount of ash of media was found in the last decade it is assumed that the increase in ash of media is a function of age, which may be explained by the demonstration of Bürger and Schlomka that any tissue of older persons becomes increased in density.

As indicated by Policard and Schultz-Brauns, it is generally agreed that the ash is made up principally of calcium, which in the incinerated preparation appears as calcium oxide. Treatment of the specimen with 2 per cent sulphuric acid produces the typical gypsum crystals of calcium sulphate, as shown in Figure 12. The material takes the hematoxylin stain as does calcium, and stains by the method of von Kossa. It is of great importance in the technical procedure that the incineration be conducted at 650° C until all organic and volatile material is removed. This can be controlled by washing with water, because if the amount of ash which remains after washing is essentially the same as before washing, it can be regarded as pure ash. As demonstrated by Policard, other minerals such as iron and magnesium are present, but these are in such small amounts that in all likelihood they can be disregarded.

As can be seen in the accompanying photomicrographs there is no doubt that muscle and connective tissue leave ash after incineration and the ash may be regarded as representing normal constituents of these tissues, as shown by Scott and by Schultz-Brauns. As indicated by the results shown in Figures 10 and 11 there is no reasonable doubt but that the elastic tissue leaves a considerable amount of ash. The origin of the elastic fibers is uncertain. Jores believed the elastica to represent the differentiation of connective tissue cell products, whereas Hueck assumed it to come from the ground substance of the connective tissue, especially that of the intima of arteries. According to Wells, hyaline substances have an affinity for calcium salts, but in spite of the possibility of adsorption of calcium, it nevertheless seems probable that normal elastic tissue contains calcium in larger quantities than other tissues. Hofmeister and Henkel maintained that compounds of calcium salts and protein do not exist in

the body. The salts are kept in solution by protecting colloids and thus the normal content of the elastica in calcium would represent only a larger concentration of calcium salts in this tissue. As is well known, such combinations are unstable and subject to precipitation by changes in hydrogen ion concentration and perhaps other physical states of the tissue. The demonstrations by Ribbert, by Klotz, and by Faber of small amounts of precipitated calcium in the arteries of young persons may be the result of precipitation due to changes of the character indicated. In outspoken intimal arteriosclerosis, where an increase in elastica can be established, it may very well be that the calcification in precipitated form depends in large part upon the high concentration of calcium in the mass of elastic tissue. Calcium salts, however, may be precipitated in the region of elastica without any preceding increase of elastica, as is true of the isolated calcification of the internal elastic membrane described by Jores. The hypothesis given above does not serve to explain all types of calcification because there are noteworthy exceptions. For example, calcification may occur in tissues in which there is little or no elastic tissue. Conversely, as in mural endocardium where elastic tissue is rich in amount, calcification may be of uncommon incidence. In idiopathic medial necrosis of the aorta of Erdheim, calcification is rare in spite of the large number of elastic fibers in this situation. The marked medial calcification in Mönckeberg sclerosis is in muscular arteries with relatively little elastica. Even in the cases reported herewith, the coronaries often showed marked hyperplasia of the elastica without any noteworthy precipitation of calcium salts. It may therefore be stated that increase in the amount of elastica alone is by no means an indication that precipitation of calcium salts will occur. The facts, however, that the elastic fibers probably contain a large amount of calcium, and that calcification in the early stages of arteriosclerosis takes place in the area where the internal elastic membrane of the artery is located, lead to the assumption that the elastic fibers play an important rôle in the calcification of arteriosclerosis.

SUMMARY AND CONCLUSIONS

1. The method of microincineration was employed in the study of the smaller divisions of the coronary arteries in 62 cases ranging in age from newborn to 79 years.

2. Ash was demonstrated in all instances, but in variable amounts. The smallest amount was found in the arteries of newborn infants. This amount gradually increased during the first, second and third decades, apparently as a corollary of normal growth. The increase in amount of ash after the third decade was definite, but was without regularity of progress. This total variation, associated with individual variation, was probably the result of arteriosclerosis in these higher age groups. It was found also that rheumatic fever nodules, and leukemic infiltrates increased the amount of ash.

3. The ash of coronary arteries is derived principally from the elastic fibers, but also in small part from the intima, media and adventitia. This should be regarded as a normal finding, but not of necessity as a precursor of arteriosclerosis.

4. The ash is composed almost entirely of calcium salts, but the manner in which these salts are bound to the elastic fibers is not known.

5. As the quantity of elastic tissue increases normally and as it increases as a part of arteriosclerosis there is an increase in the amount of ash.

6. It is suggested but not proved that alteration in quantity and quality of elastica may have some bearing upon the calcification that occurs in the course of arteriosclerosis.

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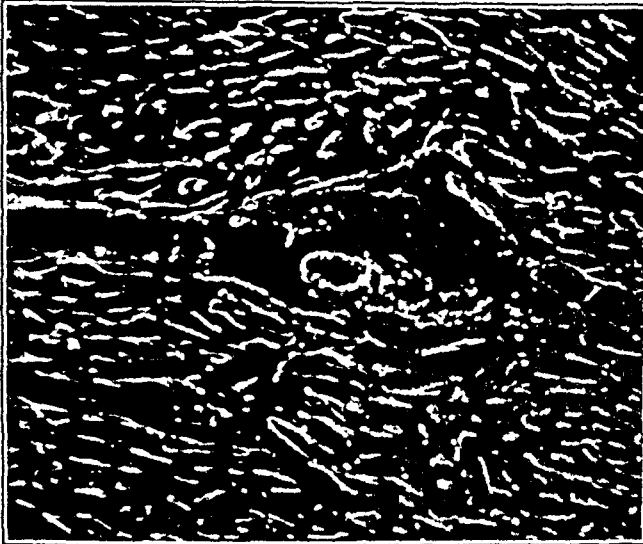
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DESCRIPTION OF PLATES

PLATE 7

All photomicrographs taken with dark-field illumination.

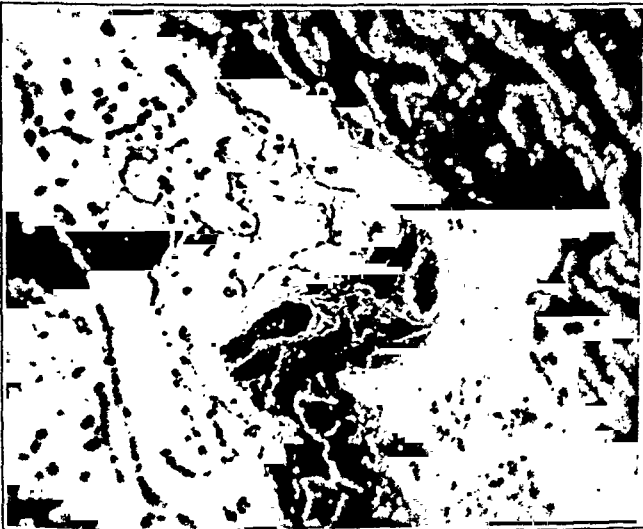
- FIG. 1. Case 5, Table I. 11 months old male. Medium-sized deep branch of left coronary. Ash forms complete ring, without distinction of three layers. $\times 150$.
- FIG. 2. Case 4, Table II. 20 year old male. Small deep branch of left coronary. Suggestion of division of ash into three layers. $\times 150$.
- FIG. 3. Case 8, Table III. 29 year old female. Two small deep branches of left coronary. Suggestion of distinction into three layers. $\times 150$.
- FIG. 4. Case 2, Table IV. 35 year old female. Medium-sized branch of right coronary artery. Ash of intima distinguished from smaller amounts of ash in media and adventitia. $\times 150$.
- FIG. 5. Case 13, Table V. 50 year old male. Medium-sized branch of right coronary. Irregular distribution of ash of sclerotic intima. Ash of media and adventitia slight in amount. $\times 150$.
- FIG. 6. Case 8, Table VI. 58 year old male. Small deep branches of right coronary. Large amount of ash from sclerotic intima. Decrease in size of lumina. $\times 150$.



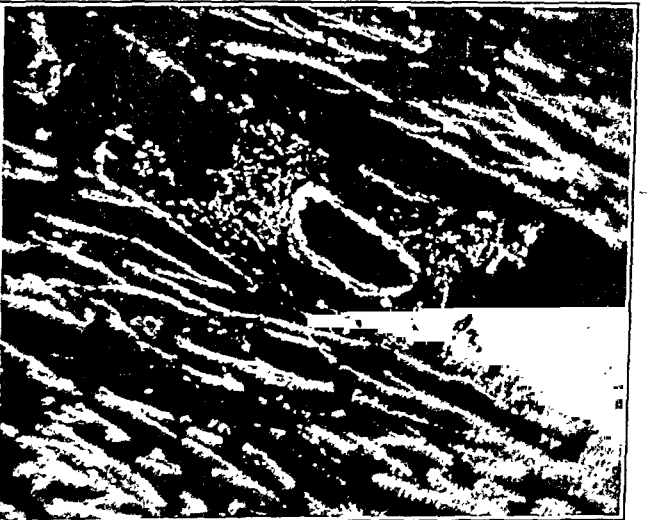
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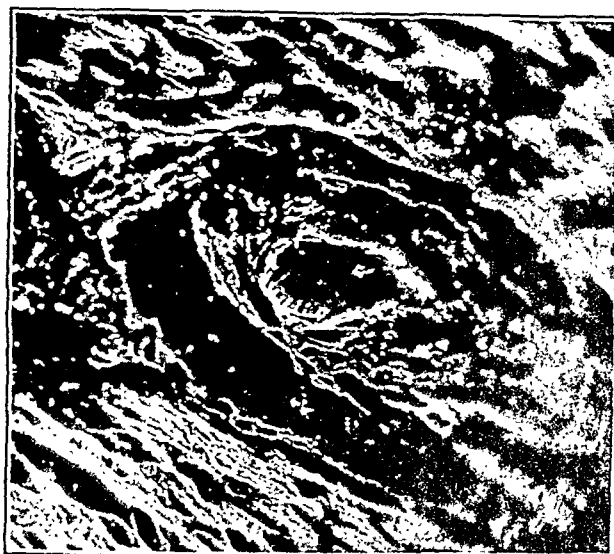
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PLATE 8

- FIG. 7. Case 10, Table VI. 60 year old male. Medium-sized branch of left coronary. Radiating arrangement of ash of sclerotic intima. $\times 150$.
- FIG. 8. Case 5, Table VII. 67 year old male. Medium-sized branch of right coronary. Inner layer of ash chiefly from intima. Note density and irregular distribution of ash. $\times 150$.
- FIG. 9. Case 3, Table VIII. 73 year old male. Small branch of right coronary. Ash of a non-sclerotic artery. Note fairly heavy deposit of ash of media and adventitia. $\times 150$.
- FIG. 10. Ash from normal aorta of a 34 year old male. The ash is principally in the media and appears to follow lines of elastica. The ash of intima is small in amount and that of adventitia still less. $\times 70$.
- FIG. 11. Ash from normal renal artery of a 34 year old male. Note the ash of the internal elastic membrane. The adventitia shows less ash than the media. $\times 70$.
- FIG. 12. Gypsum crystals of calcium sulphate in incinerated specimen of aorta, following treatment with 2 per cent solution sulphuric acid. Above is the ash in media, below are the needle-shaped crystals, and between, a clear zone where the calcium salts are in solution. $\times 150$.



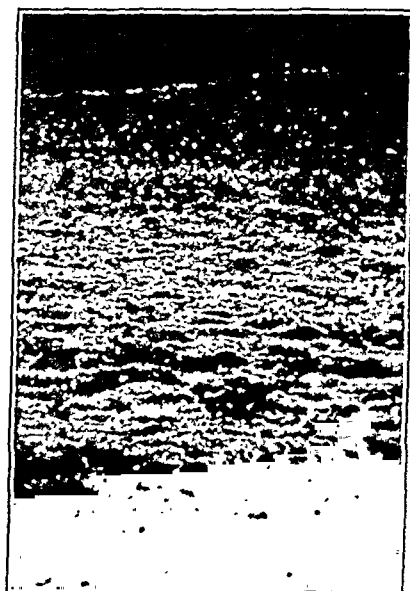
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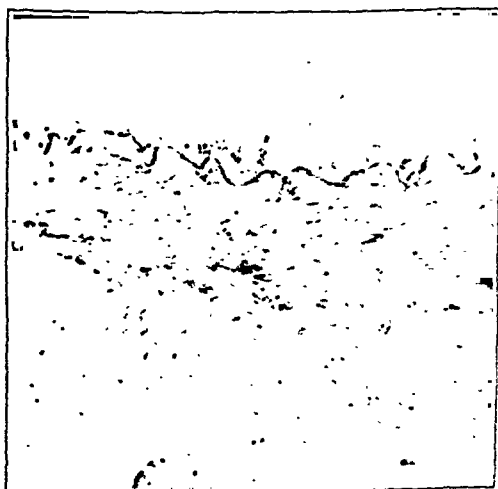
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EXPERIMENTAL FAT EMBOLISM OF THE HEART *

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In spite of numerous studies, opinions regarding the importance of fat embolism remain divided. Lehman and Moore¹ in a recent review were unable to find agreement on any phase of the problem. In previous discussions² it is repeatedly stated that fat embolism may cause death by extensive obstruction of capillaries, either in the lungs or in the brain. The pulmonary type has a rapid course with dyspnea, cyanosis, cough and signs of pulmonary edema predominating; the cerebral type has a longer course and is characterized by stupor, coma, paralyses, sphincter disturbances and so on, and by evidence of embolism of the systemic circulation, such as petechial hemorrhages in the skin, free fat globules in the retinal vessels and fat in the urine.

Other than solely mechanical effects have also been suggested. Gold and Löffler,³ working with cats, concluded that when post-traumatic fat embolism is slight death is due to simultaneously appearing shock, whereas if a large amount of fat enters the circulation death results with symptoms resembling shock. In this connection the work of Porter⁴ on the production of shock by fat embolism is of interest. Paul and Windholz,⁵ on the basis of postmortem and experimental studies, concluded that death in the cerebral form is due to injury of the brain, liver and kidneys, accompanied by symptoms of uremia. Lehman and Moore suggest that as there is little correlation between the severity of the symptoms and the amount of fat found after death physiochemical changes in the blood plasma may play an important rôle, especially as fat embolism sometimes occurs without trauma. Weimann,⁶ on the other hand, insists that changes in the brain in this form of embolism are sufficient to cause death without invoking "autotoxic processes in other organs" to explain it.

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In an extensive review Vanucci and Franceschini⁷ conclude that none of the theories — pulmonary, cerebral, renal or cardiac — adequately explain death. Although they believe that fat embolism alone can cause death in man, the part played by the fundamental disease, as well as shock accompanying injury, must be carefully considered. They also make the interesting statement on the basis of physiological experiments upon rabbits that “alterations of the function of the heart occur sooner, are more profound and more lasting than those of respiration.” As Warthin remarked in 1913,² the importance of damage to the heart in fat embolism is strangely overlooked by most students of the problem. He noted that Colley and a few other observers are the only ones who have regarded the part played by the heart as of any consequence. Warthin observed fat emboli in branches of the coronary arteries with fatty changes in the heart muscle about the plugged capillaries and arterioles. He was of the opinion that the fat droplets in the muscle fibers were derived chiefly from the emboli and only a small portion of them from invisible fat in the cells. He was greatly impressed with the effect of such changes and insisted it was proper to speak of a *cardiac-symptom complex* in fatty embolism. He stated that this is accompanied by a great fall in arterial pressure, and a corresponding rise in the venous, the heart rate being very rapid and irregular. Since Warthin's contribution others have also considered the possibility of cardiac failure in fat embolism,⁷ and have described changes in the muscle cells of the heart⁸ similar to those Warthin found. We have thought that possibly some additional light on the problem of the part played by embolism of the vessels of the myocardium would accrue from studying this phase of fat embolism experimentally.

EXPERIMENTAL METHODS

We used dogs and the method described by Rukstinat.⁹ Under ether anesthesia the thoracic cavity was opened in the left third or fourth interspace under conditions preventing infection. While the pleural cavity was open, respiration was maintained by intratracheal insufflation of air and ether. After widening the aperture with a self-retaining retractor and packing away the lower lobes of the left lung from the surface of the pericardium, the coronary arteries were exposed by a vertical incision in this sac. Into one or more of the de-

scending branches of the front coronary artery 1 to 2 cc. of dog oil were slowly injected by means of a bent hypodermic needle attached to a 2 cc. syringe. The oil was obtained by mechanically expressing the omental and subcutaneous fat of normal dogs without the use of heat or solvents. The hemorrhage from the point of puncture was controlled and the wound closed in layers. The animals were permitted to recover and live for varying periods, from 6 hours up to 30 days. They were then exsanguinated by opening the femoral arteries and veins without the use of anesthetics. Each heart was examined grossly and microscopically after hardening in formaldehyde for several days. A description was made of the exterior and of surfaces made by cutting the heart from apex to base into transverse segments several millimeters thick. Microscopic study of the changes included the examination of sections made of frozen tissue stained with Sudan III, and of others stained with hematoxylin and eosin, celloidin preparations stained by Marchi, and other methods.

GROSS ALTERATIONS

The immediate effects varied with the amount of oil injected. If more than 1.5 to 2 cc. enters the artery the heart becomes purplish and dilates, its rate first increases then diminishes, its rhythm becomes irregular, ventricular fibrillation ensues and finally the ventricles stop before the auricles. If the amount of oil entering the artery is less than 2 cc. an ill-defined area of varying size about the site of injection becomes purplish; the heart may dilate somewhat but its rate and rhythm are not appreciably changed. When the needle is withdrawn there is usually some subepicardial hemorrhage about the puncture.

The earliest change was noted at 6 hours and consisted of a deposit of fibrin on the epicardium about the injected artery and a mottling of dark to paler red-brown; beneath this the lesion continued in the wall as an irregularly but fairly well-defined region. Later, at 12 hours, the fibrinous deposit became thicker; the dark red-brown areas more sharply defined and the muscle somewhat granular and brown to pale yellow. Gradually, the dark brown places were surrounded by pale yellow-brown borders more sharply demarcated and still more granular and friable (24 hours). At 51 hours the centers of the dark brown areas were rusty brown. De-

marcation of the dark regions alternated with opaque yellow-white granular spots was increased by the appearance of pale bluish gray borders after 6 days. By the end of 10 days all these changes were well advanced. In such a heart the pericardium is adherent to the epicardium over the front upper surface about the site of injection (see Fig. 1). On surfaces made by cutting the bluish gray borders about the dark brown regions, the lines traversing these areas are depressed, translucent and broader. The central portions of these dark areas are yellow-brown. Yellow-white opaque areas are surrounded by similar borders and traversed by similar lines.

As time goes on the dark and the white areas are more and more replaced by this invading bluish gray tissue and small depressions and furrows with bluish gray translucent floors are evident externally. Later (20 days) the altered regions are converted into irregular but sharply circumscribed, soft, depressed brown areas on the surfaces made by cutting, and finally (30 days) the ventricular wall in such regions becomes considerably thinned. In a 30 day heart the pericardium is firmly adherent to the epicardium on the front surface (see Fig. 2) in several places. There are several irregular depressions on the front lower surface up to 1.5 mm. deep with bluish gray translucent floors. On surfaces made by cutting into these depressions the left ventricular wall is 3 mm. thick. Elsewhere at its thinnest portion this wall is 9 mm. in thickness. The muscle in these thinned places is almost entirely replaced by soft, dark brown, depressed tissue. There are two small areas, elsewhere on the front wall, up to 1 mm. in diameter which are soft, dark brown and depressed with puckered margins.

HISTOLOGICAL EXAMINATION

In the beginning (6 hours) there accumulate large numbers of polymorphonuclear leukocytes, lymphocytes and extravasated red blood corpuscles between the muscle fibers which are torn and separated by globules and irregular masses of fat. In the largest of such places these cells are enmeshed in a network of fibrin. Some of the muscle fibers about these lesions are already granular and hyaline with indistinct nuclei. A little later these changes become more extensive. Red blood cells, lymphocytes and polymorphonuclear leukocytes are diffusely scattered over entire sections in the inter-

stices of the muscle. Especially dense are these extravasations and accumulations beneath the endocardium. Most of the fat here is in the form of long, sausage-shaped emboli distending capillaries, following their tortuous course and filling the bifurcations with Y-shaped figures. Only where the cellular accumulation and blood extravasation are most dense have the muscle fibers become hyaline and granular with pale, indistinctly staining nuclei.

At 24 hours more of the muscle fibers become completely hyalinized or granular, increased in diameter, with nuclei which are either poorly stained and pyknotic, or disintegrated into small irregular bodies which stain deeply with basic dyes. In the fibrin adherent to the epicardium polymorphonuclear leukocytes and lymphocytes accumulate.

Gradually the altered muscle is removed and replaced by young fibrous tissue. For example, after 42 hours the granular and hyaline muscle fibers are distorted and fragmented. Closely apposed to the ends of these fibers are numerous small, multinuclear cells about 10 micromillimeters in diameter, containing up to six dark purple-staining nuclei with very pale staining cytoplasm. Among the accumulated cells, especially in the interstitium and about the capillaries, young fibroblasts with oval and spindle vesicular nuclei with numerous mitoses are present. Here and there is a nucleus in the altered muscle resembling the Anitschkow "myocyte"¹⁰ with a slightly central or eccentric, dark staining body with fine chromatin lines radiating peripherally. Many capillaries are still distended by sausage-shaped emboli of fat.

Later on, at 51 hours, the extravasated red cells are crenated and pale, the disintegrated muscle further resorbed, the accumulated cells fewer and fibroblasts more numerous. About some of the fat masses there are very minute droplets of fat in the muscle fibers (Fig. 3) as Warthin noted: "In the Marchi preparations around the blackened masses of fat are numerous muscle fibers, the sarcoplasm of which contains a dense accumulation of fine, black granules and small globules, arranged in longitudinal lines. The striations of muscle fibers with most numerous granules are absent or very faint."

At 10 days, regions of degenerated muscle cells between sausage-shaped, tortuous fat emboli remain as islets in broad bands of dense, young fibrous tissue. The resorption of the fat goes on apace (see Fig. 4). This gradual removal of the shrunken hyalinized fragments

of muscle and of fat continues (16 days) until at 20 days little else is left in the altered places except dense, vascular fibrous tissue containing free globules of fat. Oval-shaped phagocytes filled with coarse globules of fat, and others with a fine granular yellow-brown pigment, are numerous. After 30 days the fibrous tissue, still denser, almost completely replaces the muscle in one region from endocardium to epicardium (Fig. 5). But even after 30 days fat globules are still present in the fibrous tissue (Fig. 6).

DISCUSSION

Unchanged oil from canine adipose tissue was regarded as perhaps less objectionable than an oil quite foreign to dogs. The hemorrhage, infiltration with leukocytes, necrosis, appearance of fat in the muscle fibers, gross alterations of color, disappearance of necrotic tissue and cicatrization have all been described by others.^{10, 11} The differences we observed in these infarcts produced by canine fat were, in some particulars, related to the physical nature of the material used. The oil spread out in the capillaries in a manner similar to the distribution Wearn¹² obtained of material he injected to study the capillary bed of the myocardium. The small size of the infarcted regions we produced in dogs' hearts with oil may be due to the liberal anastomosis Wearn demonstrated, and to the absence in the preparations we studied of vessels larger than capillaries plugged by the oil. Apparently infarction followed permanent blocking with oil of all the capillaries in certain limited portions of the myocardium, rather than occlusion of some large or medium-sized artery, as takes place naturally with coronary thrombosis and embolism, and which has usually resulted in most of the occlusion experimentally produced. This contrast between these oil infarcts and the usual type in which vessel blockade is confined to the periphery is particularly noteworthy, notwithstanding the significance of the dissimilarity is possibly limited to infarcts produced experimentally. The depressions found on the outside of the hearts of dogs 16 to 30 days after injection of the oil suggest that the capillaries where the oil lodged belong to the perpendicular branches of the subepicardial division of the arteries described by Whitten.¹³

We were surprised to find some of the oil still present after 30 days in the cicatrices. In preparations examined 6 days after injection

there were minute drops of oil in large phagocytic cells; evidently it is disposed of very slowly. About some, but not about all of the emboli of fat, the adjacent muscle fibers were fatty, as noted by Warthin. We are unable either to confirm or refute his opinion that the fat in such fibers was embolic and had accumulated in the muscle after passing through the capillary walls. Some of the droplets were larger than those found in the usual fatty degeneration of the myocardium, the "herring-bone" or "tabby-cat" fatty change, for example. It may not be proper to assume that scars such as we studied would be widely disseminated throughout the myocardium if the oil had passed out through both coronary arteries so as to attain a wide distribution in the heart muscle. On the other hand, Vance¹⁴ has reported minute streaky or flame-like hemorrhages several millimeters long scattered throughout the human myocardium from fat embolism due to automobile injuries. He also states that permanent scars due to "this process" have not been described. Perhaps pulsations of the human heart so aid in removal of fat emboli that minute focal scars do not follow, or possibly fat embolism should be added to the long list of causes now held responsible for disseminated minute scars throughout the myocardium, the so-called interstitial myocarditis.

A third possibility is that death occurs too soon for such scattered scars to develop throughout the myocardium, that with fat embolism sufficient to produce these focal lesions in the heart, other organs, and especially the brain, are also involved and patients so afflicted die at the height of systemic generalized fat embolism.

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DESCRIPTION OF PLATES

PLATE 9

FIG. 1. (10 days.) The region of infarction is easily followed from one to another in these segments of the ventricles. In the more apical it is darker than the rest of the myocardium; toward the base beginning cicatrization has made it paler. Part of the pericardial sac with a little of the left lung is bound fast by fibrous adhesions to a portion of the periphery of the first four segments.

FIG. 2. (30 days.) Considerable of the outside of the heart is covered by adherent pericardium. Near the apex is one deep dent. Above and to the left several smaller depressions are near the right border. These are all infarcts partly cicatrized.



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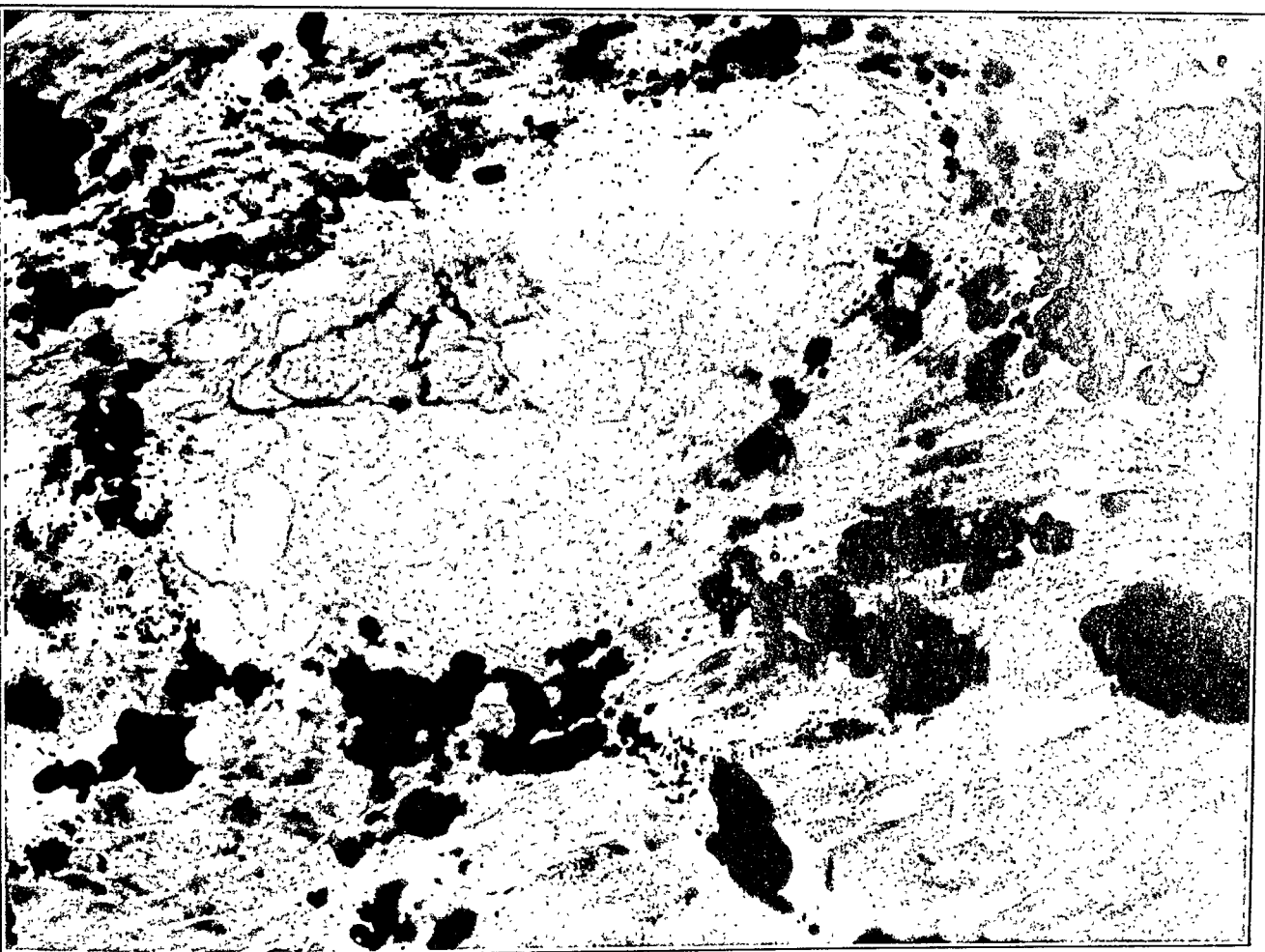


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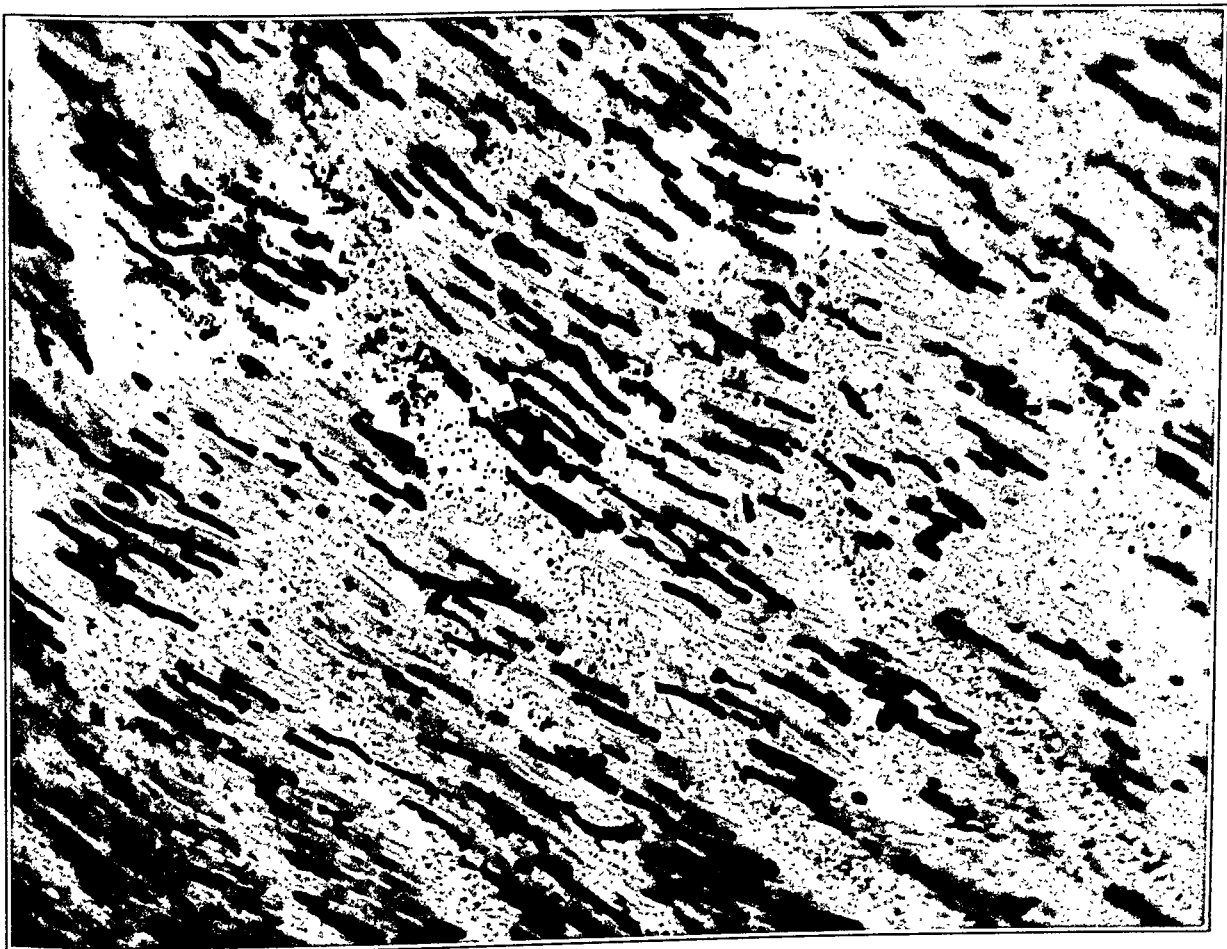
PLATE 10

FIG. 3. (51 hours.) The large pear-shaped region is filled with blood in a mesh-work of fibrin. The injected oil is black as are also fine droplets of fat in the muscle fibers. Marchi and Bismark brown.

FIG. 4. (10 days.) Extensive occlusion of capillaries by sausage-shaped emboli of dog oil. The torn places, most of them extending up and to the left, are filled with erythrocytes and among these are minute drops of fat in phagocytes. Marchi and Bismark brown.



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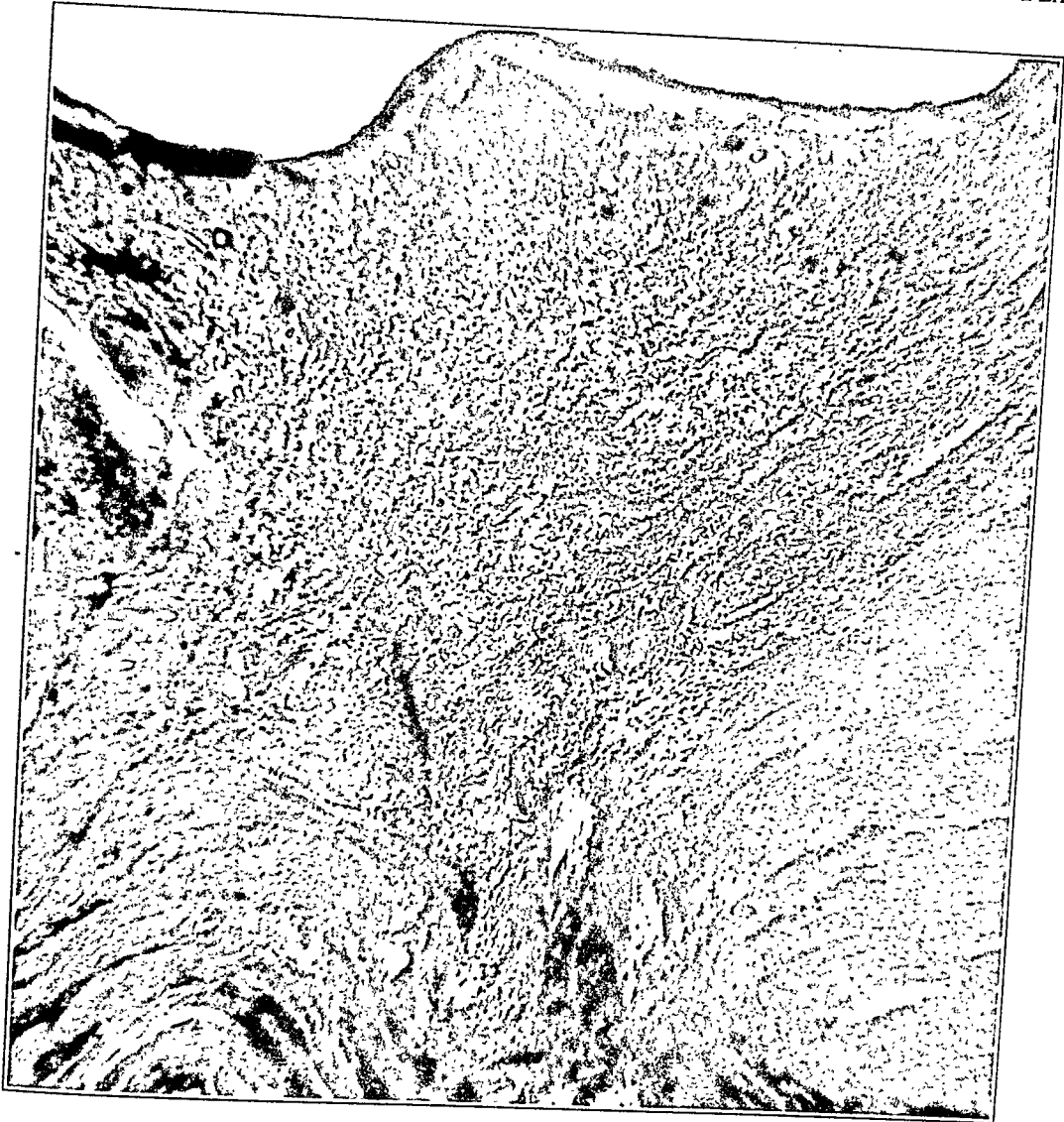


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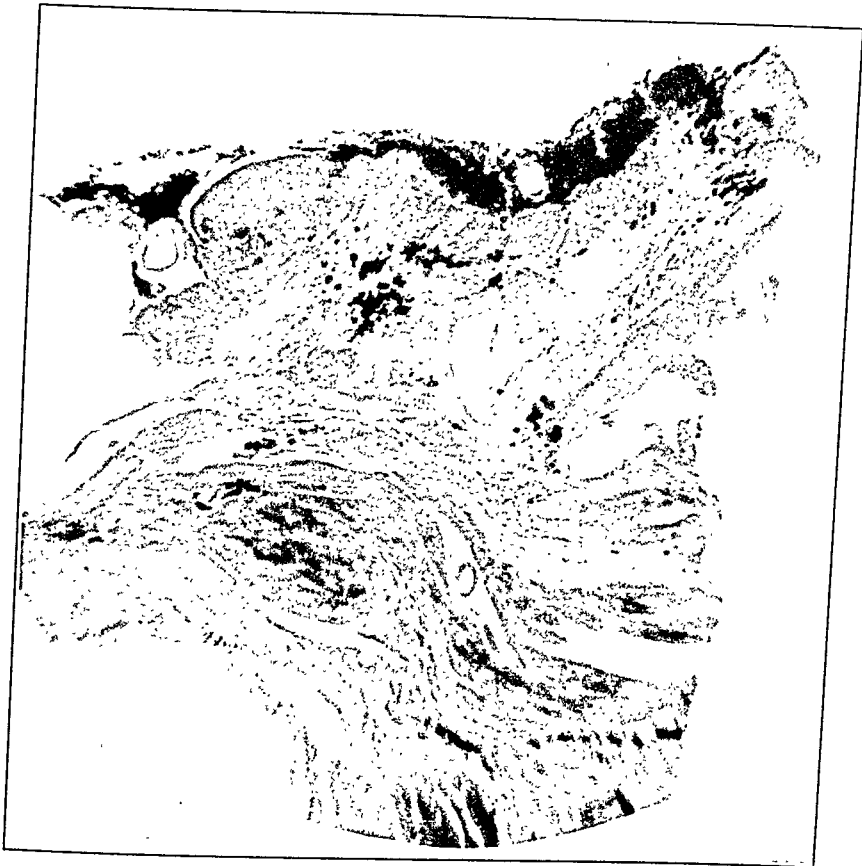
PLATE II

FIG. 5. (30 days.) A low power illustration of one of the infarcts with myocardium replaced by highly vascular granulation tissue. The wall of the left ventricle here was only 3 mm. thick. Hematoxylin and eosin.

FIG. 6. (30 days.) Large masses of dog oil in granulation tissue well advanced toward cicatrization.



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BODY LENGTH AND ORGAN WEIGHTS OF INFANTS AND CHILDREN *

A STUDY OF THE BODY LENGTH AND NORMAL WEIGHTS OF THE
MORE IMPORTANT VITAL ORGANS OF THE BODY BE-
TWEEN BIRTH AND TWELVE YEARS OF AGE

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Owing to the meager data regarding weights of normal organs of infants and children, it seemed advisable to make use of our records for this purpose. Our intention was only to do sufficient work to check up on published tables. Impetus carried us beyond this point and led us to examine all our available data and then to prepare for our own use tables and graphs for handy reference. These we have used to our own satisfaction for three years and we now publish them for what they may be worth.

The graphs are self-explanatory. After due consideration we decided not to elaborate upon them, believing that the information as presented is amenable to such mathematical treatment as may seem desirable by persons interested. However, the data employed may be obtained from us, as well as those employed for graphs omitted from this article, in which the weights of the same organs have been plotted according to body length. For the benefit of those having use for the data included in the graphs, we include information about the methods of selection of material.

At the Children's and Infants' Hospitals 2287 autopsy records were examined, of which 1043, or 45.6 per cent, were selected. Each selected autopsy contributed a normal weight of at least one vital organ. Similarly, 7194 autopsy records were examined at the Boston City Hospital, of which 155 or 2.15 per cent, between the ages of 6 and 12 years were selected. These latter autopsies supplemented the data from the Children's and Infants' Hospitals, making a total of 1198. Upon these the substance of this study is based.

* Received for publication May 31, 1932.

The following is a tabular summary of the records examined:

Hospital	Age	No. records examined	Years cases selected	No. selected	Per cent selected	Total per cent selected
C. H. and I. H.	Birth to 6 yrs.	2287	1914-1929	1043	45.6	87
B. C. H.	6 to 12 yrs.	7194	1896-1929	155	2.15	13
Total		9481		1198	47.75	100

The information gained from each selected autopsy record was tabulated in the following manner:

Age period	Autopsy No.	Age	Body length	Heart	Lungs		Spleen	Liver	Kidneys		Brain
					Right	Left			Right	Left	
6 mos.	26-130	6 mos.	cm. 58	gm. 34	gm. 45	gm. 32	gm. 16	gm. 225	gm. 26	gm. 23	Restricted

Similarly the body length in centimeters and the weight of the various organs in grams were catalogued under the particular age period to which the case belonged. If an organ was recorded as diseased or otherwise abnormal, it was so marked and later rejected. When no demonstrable pathological changes were described, the weight of the organ was accepted as normal. Prematurity was sufficient reason for rejection.

The selected cases were separated according to age into thirty-two periods, *e. g.*, birth to 3 days (inclusive), 3 to 7 days, 1 to 3 weeks and so on (Table I). The average weight in grams of each organ and the average body length in centimeters for each age period were then computed. The figures are given in Table I. An attempt was then made to show graphically how the various figures for each organ at every age period were distributed above and below the computed arithmetical mean. Thus, the upper and lower limits of normal were ascertained. For each age period along the growth graph of each organ there was charted the number of organ weights which fell within 5 grams (in the lungs, 10 grams; in the liver and brain, 50 grams, and so on) of each other, above and below the mean weight.

A similar graphic chart was constructed for the body length (Table II). Each organ weight chart presents two graphs; a lower general growth graph between birth and 12 years, and a detailed upper

graph between birth and 2 years. Both graphs give the mean weight of the organ at various age periods, with a perpendicular scale showing the number and distribution of the individual weights which yielded the arithmetical mean. The upper and lower weight limits as depicted on the perpendicular scales are arbitrary and not actual values within which actual weights are grouped. A sample working sheet of the heart at the age period of 1 to 3 weeks is shown as follows:

Grams	31	26	21	16	11	6
Instances	2	12	21	7	1	

Here the upper line of figures represents the arbitrary weight of the heart distributed at 5 gram intervals from 31 down to 6 grams. The lower line represents the number of individual weights which fall within each 5 gram interval. The actual weights within each interval were represented on the working sheets as follows:

GRAMS	INDIVIDUAL WEIGHTS	TOTAL WEIGHTS
31-26	30, 28	2
26-21	21, 22, 25, 25, 25, 23, 23, 23, 25, 25, 23, 22	12
21-16	20, 20, 20, 20, 20, 20, 18, 18, 17, 19, 18, 18, 18, 18, 18, 18, 18, 17, 17, 17, 17	21
16-11	12, 14, 14, 14, 15, 15, 15	7
11- 6	10	1
Total No. of Weights		43
Total Weight		835 grams
Mean Weight.....		19 grams

The lowest arbitrary figure in this sample sheet is 6 grams, although the lowest actual weight is 10 grams, which falls in the interval between 6 and 11 grams. Likewise there are seven cases between 11 and 16 grams, and so on. A similar system was used in the charts representing body length and the weights of the other organs, except that in the lungs cases were grouped within intervals of 10 grams, and in the liver and brain 50 grams.

On a single chart (Chart 10) the general growth graphs of all organs and body length are represented. The ordinates and abscissae are relabeled so that all graphs could be included. The graphs for the left lung and the left kidney are omitted because they correspond in general to the graphs for the right lung and kidney. No figures are available for the normal weights of lungs between 11 and 12 years of age.

Because there were such wide variations, as shown in the charts, above and below the calculated arithmetical mean, another group of charts was prepared representing a correlation between organ weight and body length in order to show the weights of organs at any given body length. The same method used above in calculating the organ weight at each age period was employed. The weights were grouped within intervals of 5 centimeters from 45 to 140 centimeters of body length. Having calculated the mean weight of each organ at various height levels, charts were drawn and the individual weights were grouped as before on an arbitrary vertical scale above and below the calculated mean weight of the organ. A similar, but slightly less marked variation in the upper and lower limits of normal occurred in the second set of charts (not reproduced here).

A comparison of our figures with those of other authors is given below:

WEIGHT OF HEART

<i>Age Period</i>	<i>Boyd¹</i>	<i>Authors'</i>
Birth	20.6 gm.	17 gm.
1½ yrs.	44.5 "	52 "
3 yrs.	60.2 "	59 "
5½ yrs.	72.8 "	85 "
10½ yrs.	122.6 "	122 "

WEIGHT OF BRAIN

<i>Age Period</i>	<i>Feer²</i>	<i>Authors'</i>
Birth	370 gm.	335 gm.
2 mo.	460 "	489 "
4-6 mo.	600 "	644 "
11-12 mo.	850 "	925 "
2 yrs.	970 "	1064 "
3 "	1100 "	1141 "
4 "	1190 "	1191 "

NORMAL WEIGHTS OF ORGANS OF NEW-BORN

<i>Organ</i>	<i>Craig³</i>	<i>Authors'</i>
Brain	385 gm.	335 gm.
Heart	24 "	17 "
Kidneys	23 "	right 13 "
Liver	118 "	78 "
Lungs	58 "	right 21 "
Spleen	8 "	8 "

BODY LENGTH

<i>Age Period</i>	<i>Feer</i>	<i>Holt & Howland⁴</i>	<i>Authors'</i>	<i>Age Period</i>	<i>Feer</i>	<i>Holt & Howland⁴</i>	<i>Authors'</i>
Birth ...	50 cm.	52.0 cm.	49 cm.	6 yrs. ...	109 cm.	... cm.	109 cm.
6 mo. ...	"	67.4 "	62 "	7 " ...	115 "	... "	113 "
12 mo. ...	75 "	75.0 "	73 "	8 " ...	120 "	... "	119 "
18 mo. ...	"	80.0 "	78 "	9 " ...	125 "	... "	125 "
2 yrs. ...	85 "	85.1 "	84 "	10 " ...	130 "	... "	130 "
3 " ...	93 "	94.0 "	88 "	11 " ...	135 "	... "	135 "
4 " ...	99 "	... "	99 "	12 " ...	140 "	... "	139 "
5 " ...	104 "	... "	106 "				

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TABLE I. ORGAN WEIGHTS

*Children's and Infants' Hospital Series**Supplemented Between 6 to 12 Years by Data from the Boston City Hospital*

Age	Body length	Heart	Lungs		Spleen	Liver	Kidneys		Brain
			Right	Left			Right	Left	
	cm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Birth-3 days	49	17	21	18	8	78	13	14	335
3-7 "	49	18	24	22	9	96	14	14	358
1-3 wks.	52	19	29	26	10	123	15	15	382
3-5 "	52	20	31	27	12	127	16	16	413
5-7 "	53	21	32	28	13	133	19	18	422
7-9 "	55	23	32	29	13	136	19	18	489
9-3 mos.	56	23	35	30	14	140	20	19	516
4 "	59	27	37	33	16	160	22	21	540
5 "	61	29	38	35	16	188	25	25	644
6 "	62	31	42	39	17	200	26	25	660
7 "	65	34	49	41	19	227	30	30	691
8 "	65	37	52	45	20	254	31	30	714
9 "	67	37	53	47	20	260	31	30	750
10 "	69	39	54	51	22	274	32	31	809
11 "	70	40	59	53	25	277	34	33	852
12 "	73	44	64	57	26	288	36	35	925
14 "	74	45	66	60	26	304	36	35	944
16 "	77	48	72	64	28	331	39	39	1010
18 "	78	52	72	65	30	345	40	43	1042
20 "	79	56	83	74	30	370	43	44	1050
22 "	82	56	80	75	33	380	44	44	1059
24 "	84	56	88	76	33	394	47	46	1064
3 yrs.	88	59	89	77	37	418	48	49	1141
4 "	99	73	90	85	39	516	58	56	1191
5 "	106	85	107	104	47	596	65	64	1237
6 "	109	94	121	122	58	642	68	67	1243
7 "	113	100	130	123	66	680	69	70	1263
8 "	119	110	150	140	69	736	74	75	1273
9 "	125	115	174	152	73	756	82	83	1275
10 "	130	116	177	166	85	852	92	95	1290
11 "	135	122	201	190	87	909	94	95	1320
12 "	139	124	93	936	95	96	1351

TABLE II. BODY LENGTH

*Children's and Infants' Hospitals Series**Supplemented Between 6 to 12 Years by Data from the Boston City Hospital*

Age	Body length in cm.	No. cases	Age	Body length in cm.	No. cases
Birth-3 days	49	15	14 mos.	74	30
3-7 "	49	15	16 "	77	26
1-3 wks.	52	29	18 "	78	22
3-5 "	52	30	20 "	79	21
5-7 "	53	16	22 "	82	11
7-9 "	55	40	24 "	84	20
9-3 mos.	56	53	3 yrs.	88	36
4 "	59	36	4 "	99	14
5 "	61	41	5 "	106	16
6 "	62	43	6 "	109	36
7 "	65	35	7 "	113	41
8 "	65	29	8 "	119	21
9 "	67	18	9 "	125	27
10 "	69	20	10 "	130	20
11 "	70	20	11 "	135	17
12 "	73	11	12 "	139	16

In all charts of weights (Charts 2 to 9 inclusive) the column of figures on the left refers to grams, that on the right in Chart 10, to centimeters.

In Chart 1, the column on the left refers to centimeters. In all charts, the upper line of horizontal figures is the age scale for the upper graph, the lower line for the lower graph.

BODY LENGTH

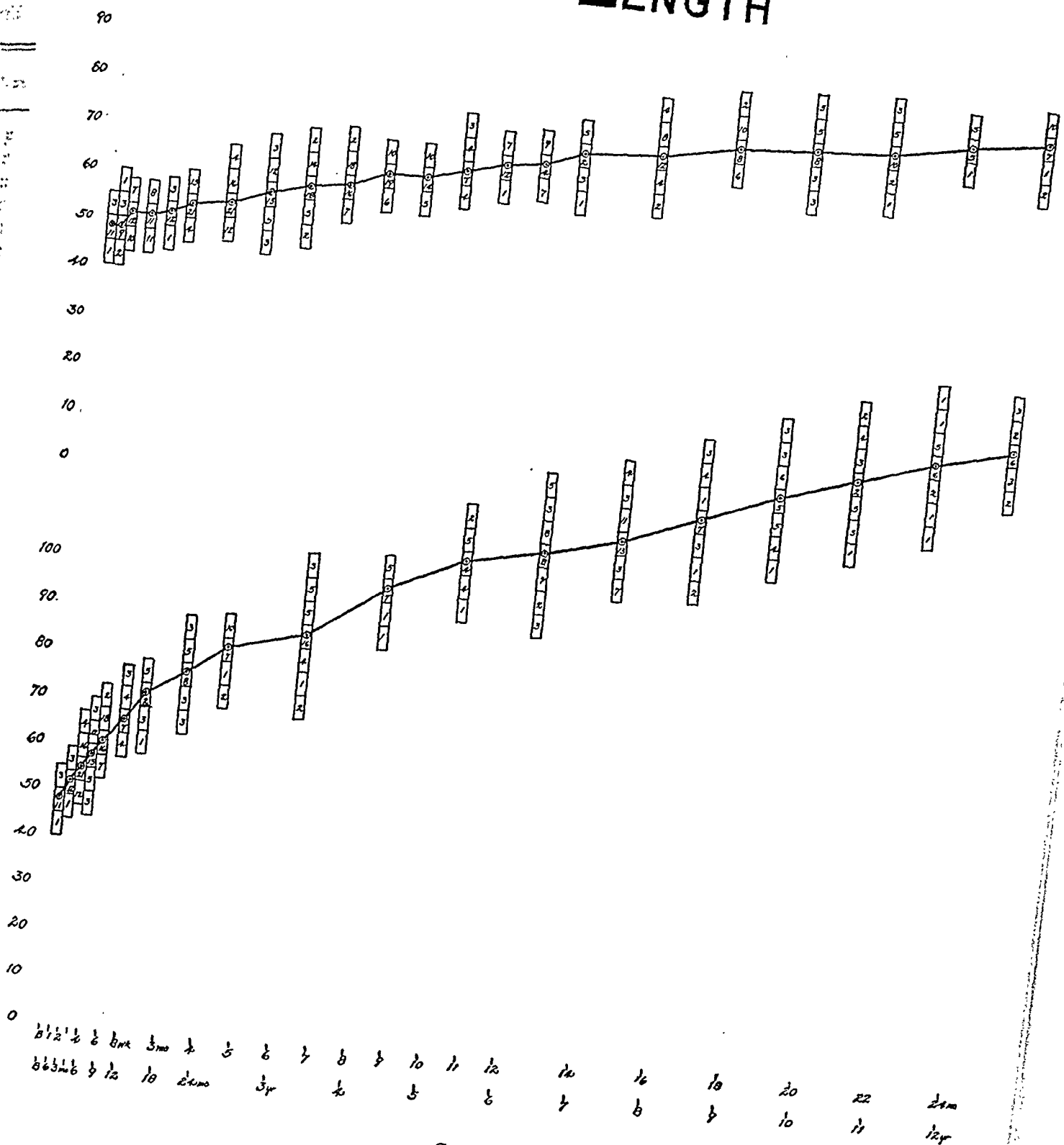


CHART I

HEART

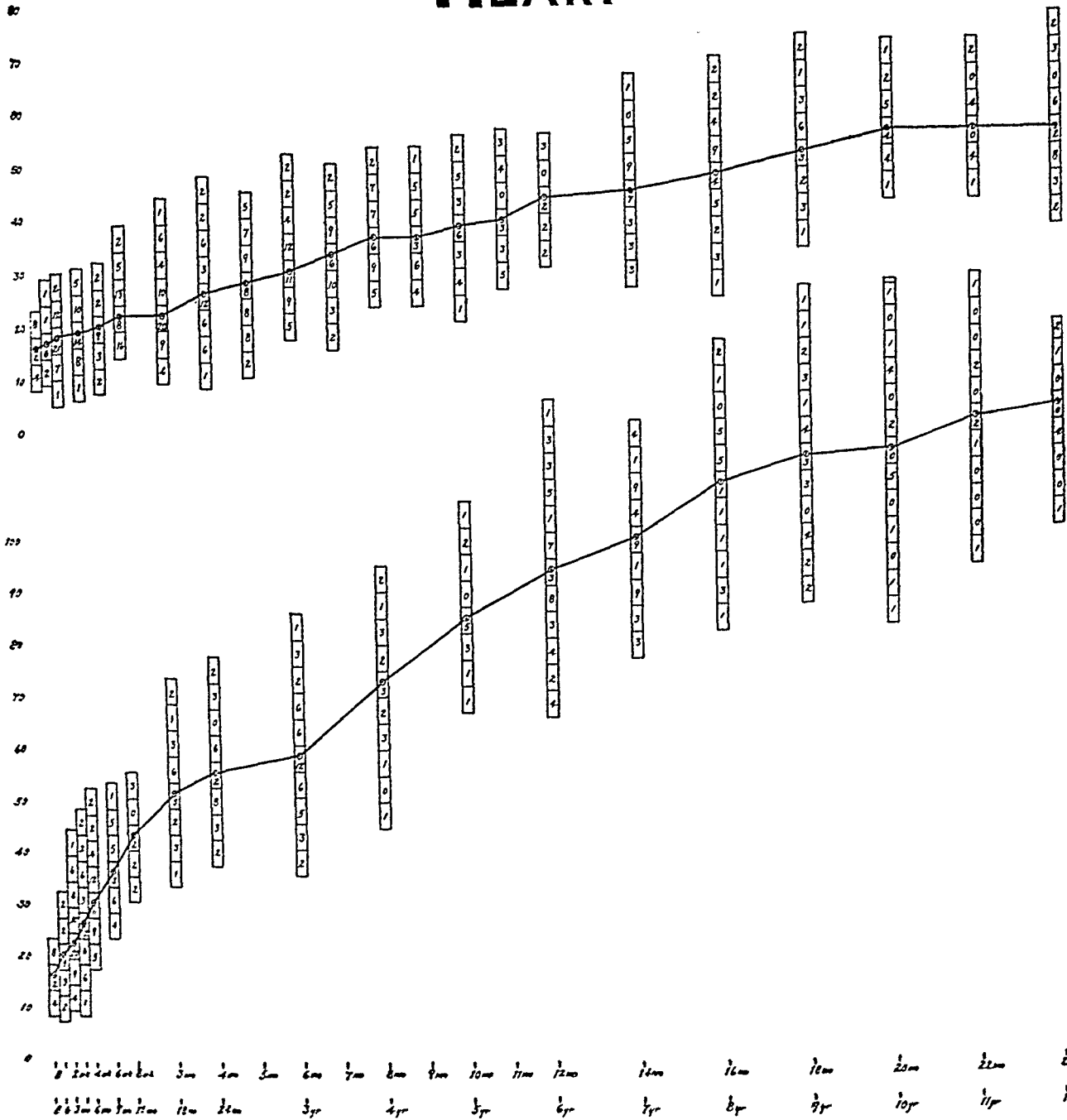


CHART 2

RIGHT LUNG

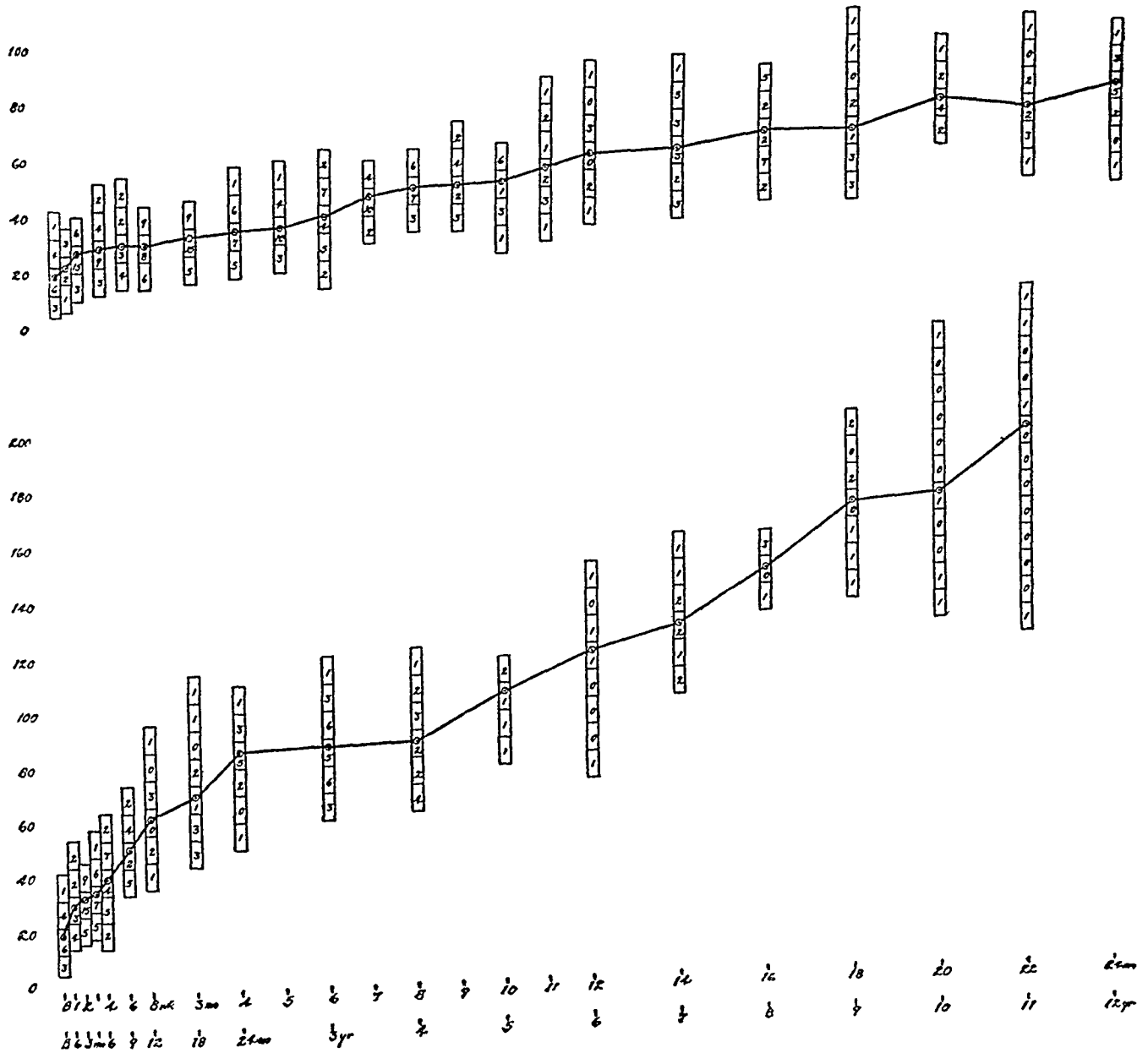
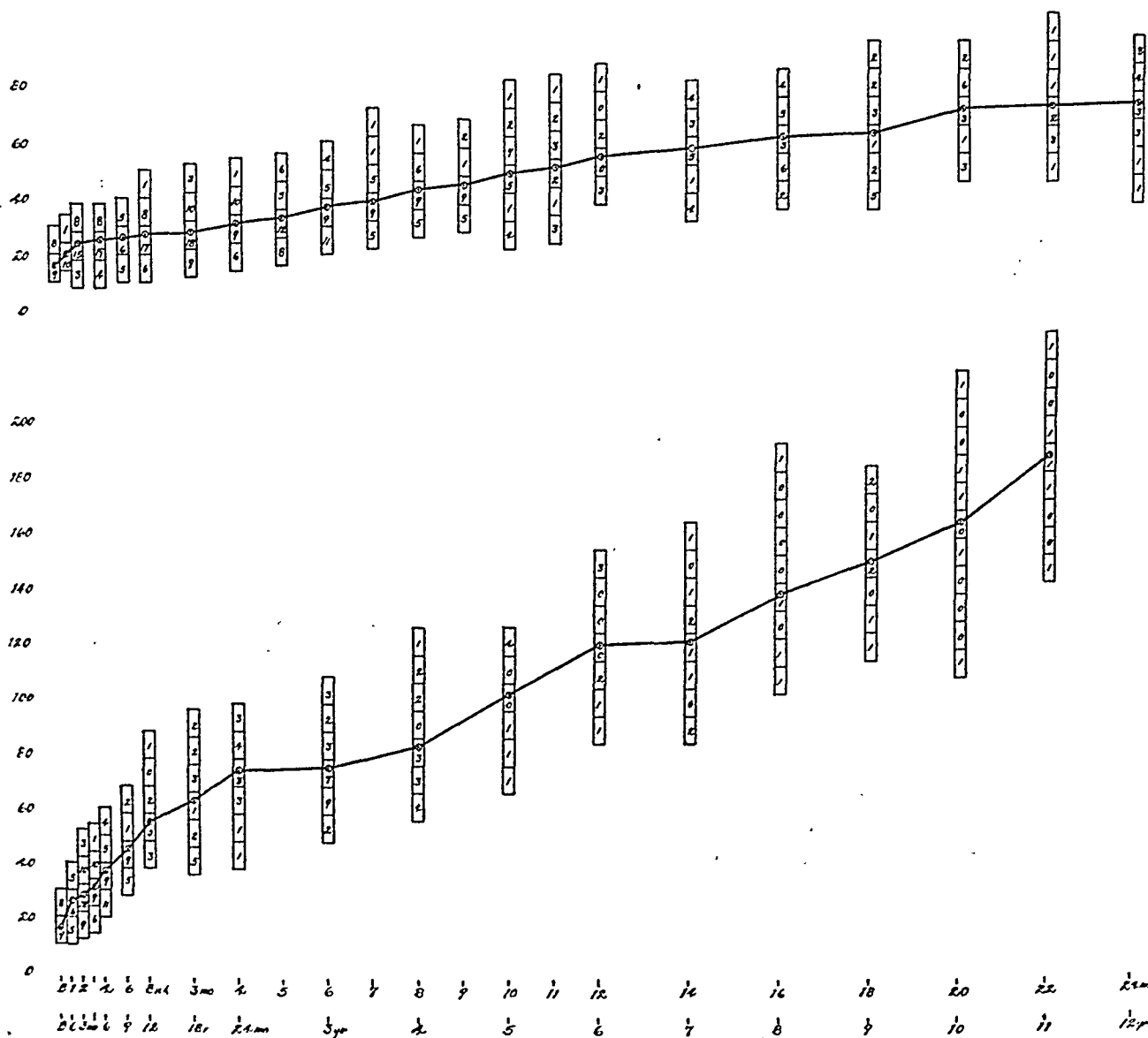


CHART 3

LEFT LUNG



SPLEEN

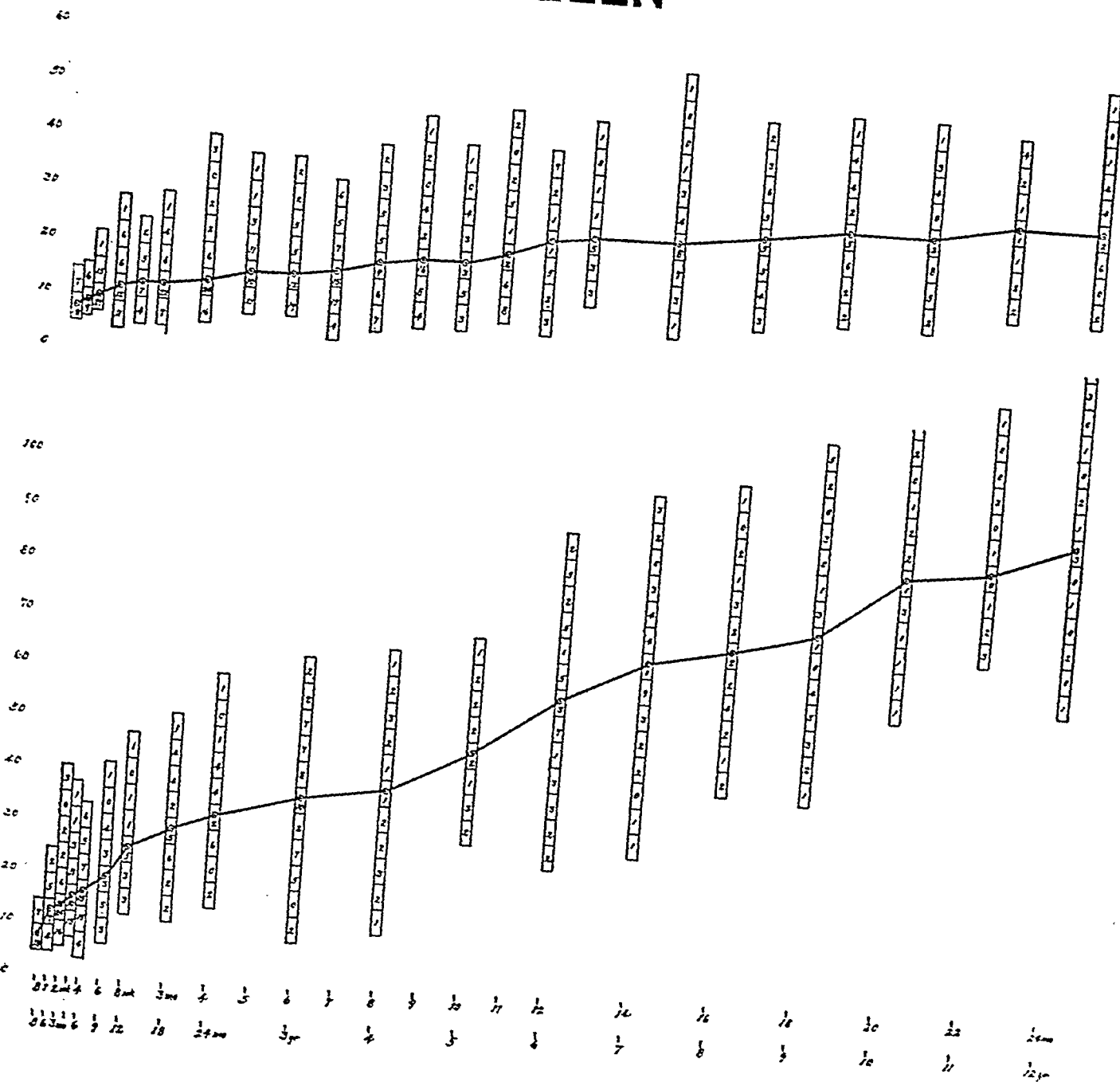


CHART 5

LIVER

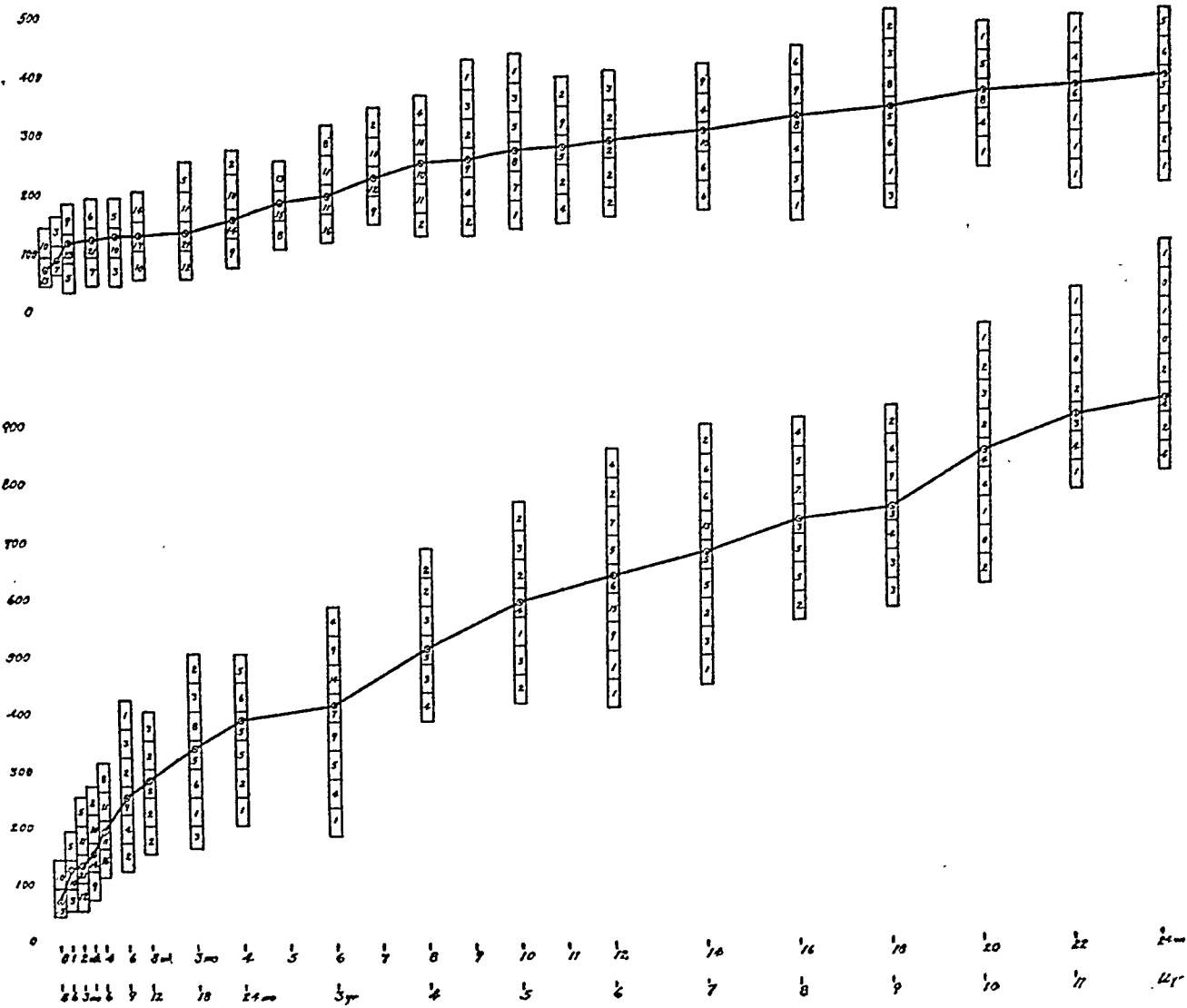


CHART 6

RIGHT KIDNEY

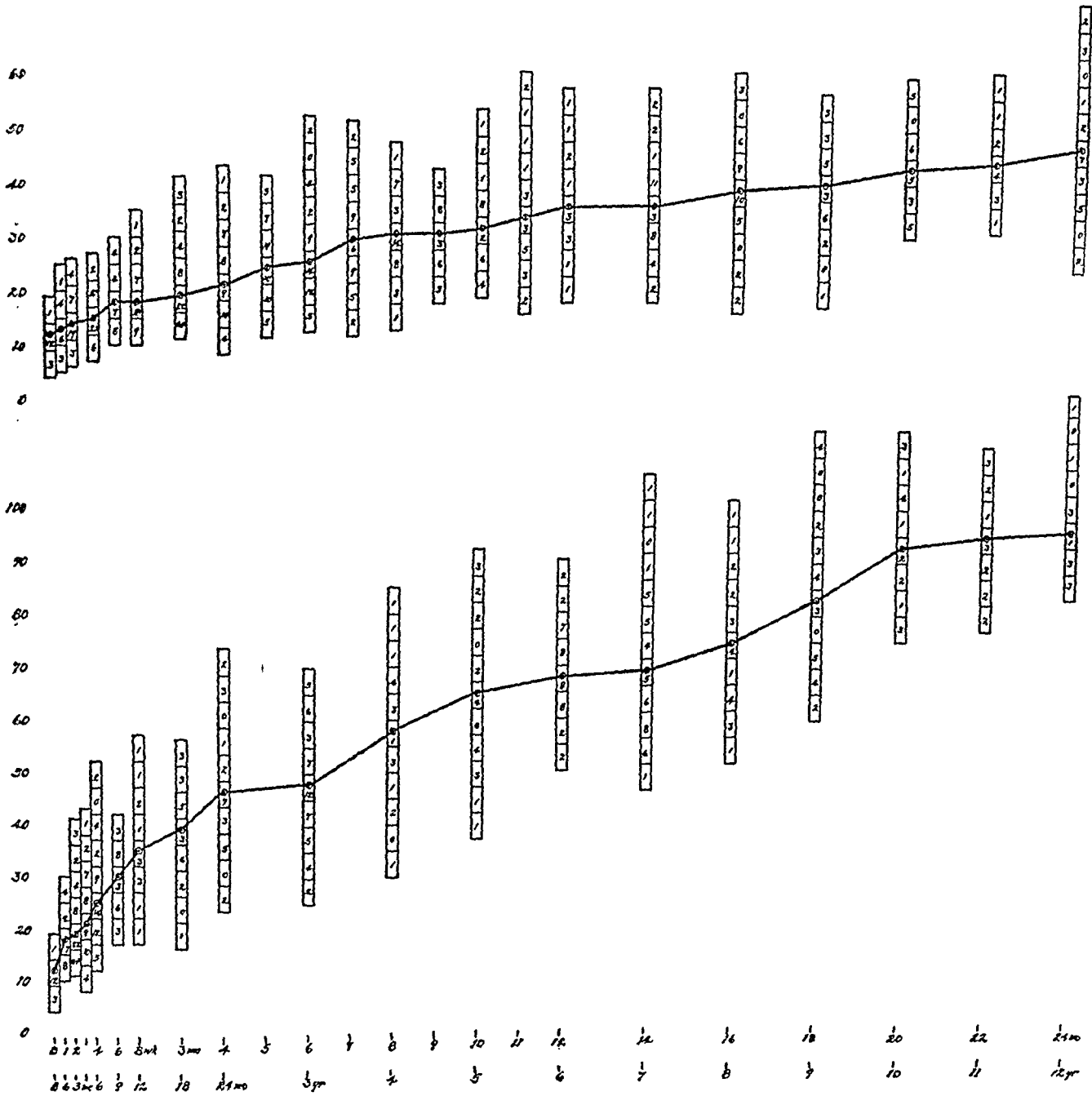


CHART 7

LEFT KIDNEY

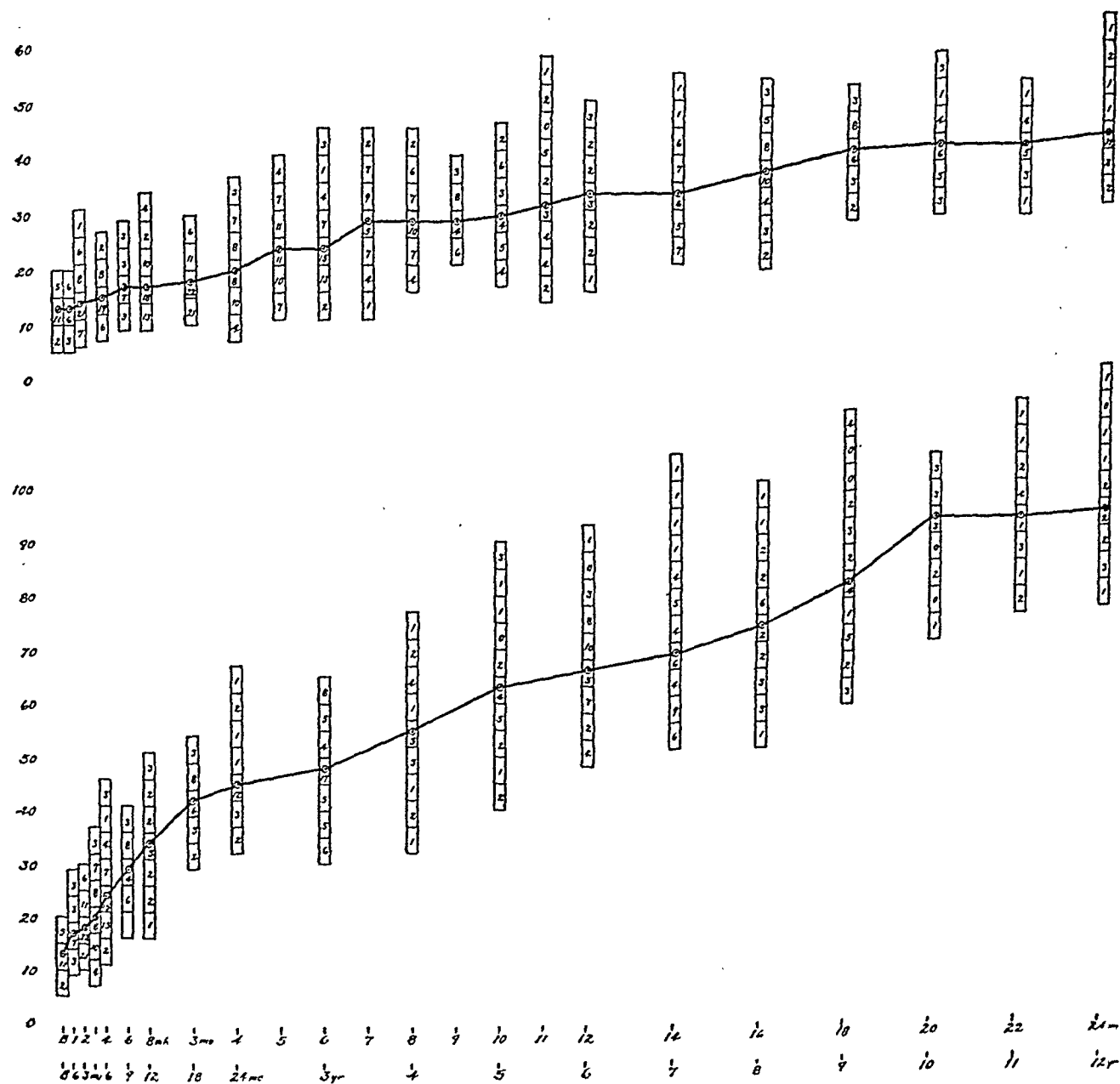


CHART 8

BRAIN

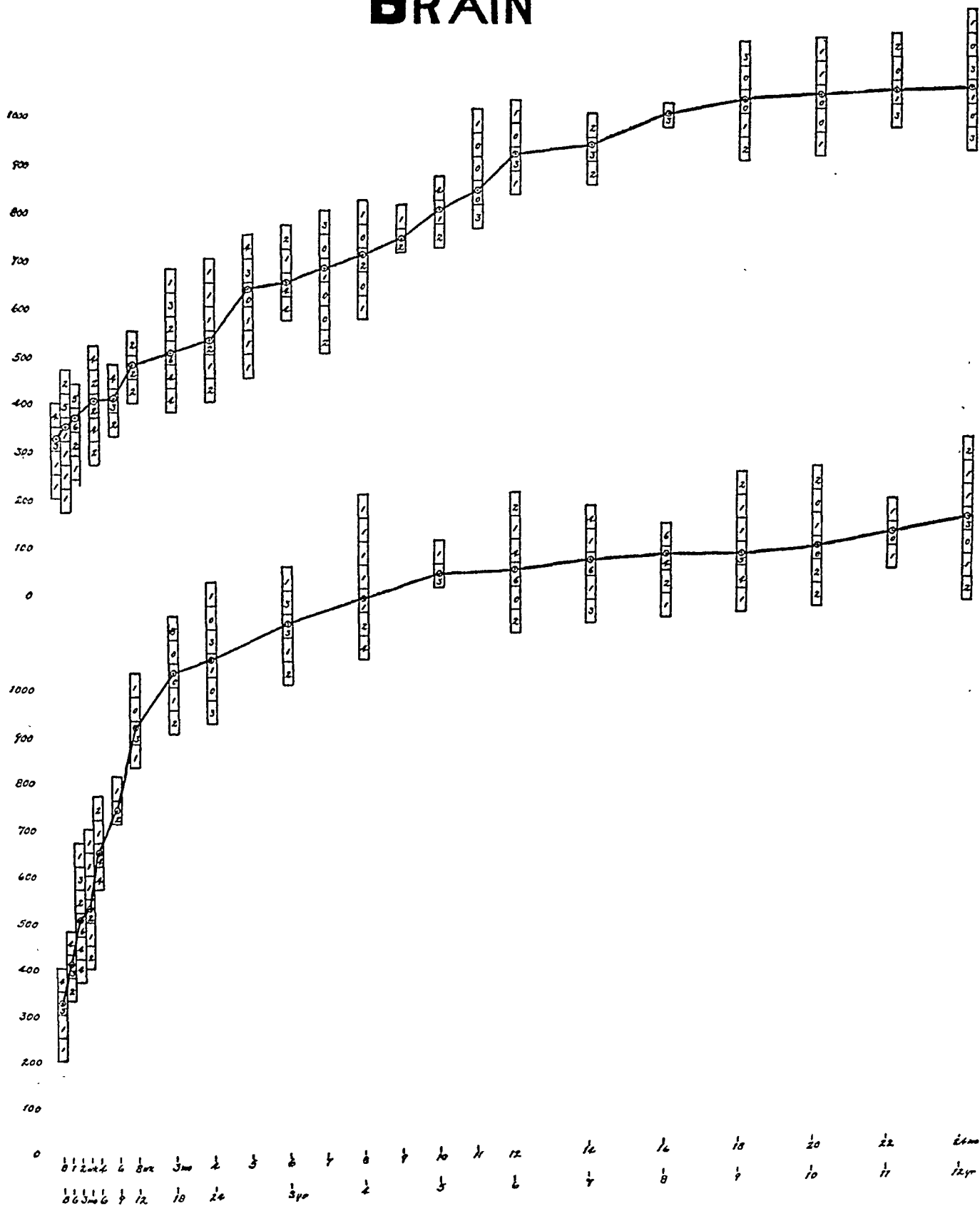


CHART 9

ALL ORGANS

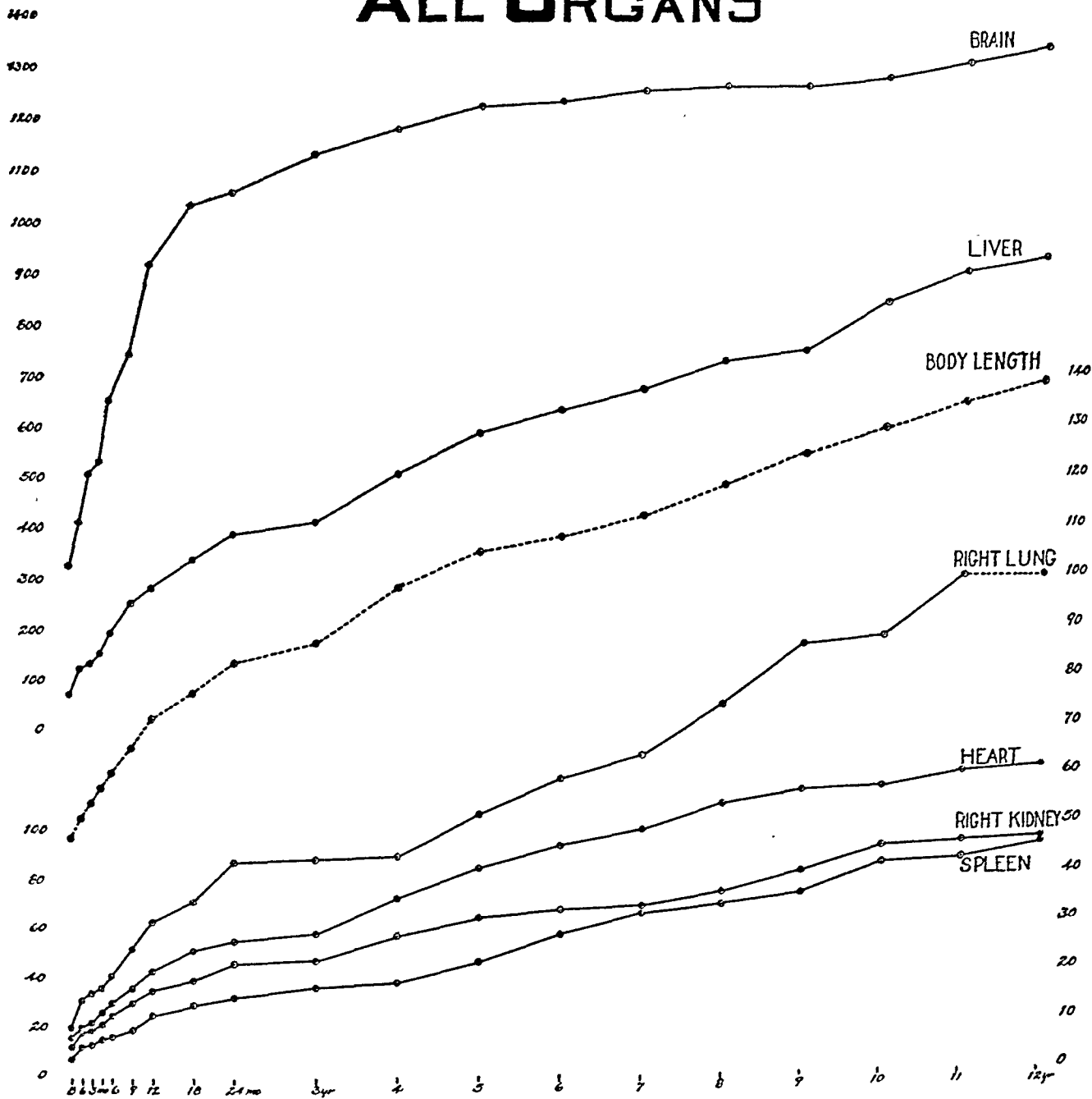


CHART 10

MULTIPLE LIVER ABSCESES CAUSED BY A LEPTOTHRIX, WITH A REVIEW OF LEPTOTHRICAL INFECTION *

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Mycotic abscesses of the liver are quite uncommon, and of those encountered the etiological agent is usually an actinomyces.¹ As yet there are no cases on record of liver abscesses produced by a cladotrix or leptothrix. The case herewith reported is one in which multiple abscesses of the liver were found to be caused by a leptothrix which was successfully grown on artificial culture media.

The members of the leptothrix group of microorganisms have a very low pathogenicity, but have been grown on artificial culture media only rarely. According to Hunt Müller² they are frequently found as saprophytes in the human mouth. Von Beust³ found leptothrices regularly in gingivitis and stomatitis, but their significance is uncertain. Mendel⁴ isolated an anaerobic leptothrix from a case of alveolar pyorrhea and found it pathogenic for animals. Infection of the lachrymal canals by leptothrices with production of Parinaud's conjunctivitis is fairly common, and infection of the pharynx with these microorganisms is even more so; in the latter case a pseudo-membrane closely resembling that seen in diphtheria is formed which may be very resistant to treatment, lasting for months without causing much inconvenience.²

In 1896 Flexner⁵ reported the isolation of a pathogenic leptothrix from a rabbit which died five days after parturition. Acute pleuritis, pericarditis and pneumonia were associated with acute endometritis, all caused by the same organism. Cultures were obtained only by the use of fresh tissue, fetal tissue giving the best results. The organism was highly pathogenic for rabbits. A summary of the literature relating to cladotrix and leptothrix infections concludes his article.

Follet and Sacquépée⁶ in 1902 reported the isolation of a leptothrix from the blood stream and from the vesicles of a patient with an atypical zona. The spinal fluid was normal. Their organism

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seems to have been a cladothrix, for they saw false branching occasionally.

In 1901 Blumer and MacFarlane⁷ reported a series of sixteen cases of noma following an epidemic of measles in an orphan asylum. Nine cases were examined bacteriologically, and in all a leptothrix was found. In early cases few other organisms were present, but later secondary invaders were abundant. The organism stained poorly by Gram's method but did not decolorize. The leptothrix could not be cultured.

In 1905 Mathews⁸ reported a case of localized empyema due to a leptothrix. The organism appeared as free filaments, and more abundantly in colonies forming small yellowish granules. The filaments varied in length, stained readily but unevenly, showed irregular thickening, and their ends were often slightly swollen. These ends stained more deeply and tended to be Gram-positive, while the rest of the filament was Gram-negative. Cultures failed to grow and guinea pig inoculation with pus was fruitless.

In 1914 Schottmüller⁹ isolated an organism from the blood of a woman who had been bitten by an African squirrel and subsequently developed pustules all over the body. Smears of the pus showed bacillary forms. Blood agar plates smeared with pus showed no growth grossly, but stained smears showed numerous sinuous filaments. Blood culture also showed no gross growth, but smears showed short filaments. Subcultures of these cultures showed no growth. Schottmüller calls this organism a streptothrix instead of a leptothrix because of "die schön geschwungene und gewundene Wuchsform in der Eiterkultur," although no branching was seen.

Deussing¹⁰ in 1920 reported a case of leptothrix angina accompanied by infection with a diplococcus and complicated by glomerulonephritis. The nephritis he attributed to the coccus, but Huntzmüller feels that it may have been due to the leptothrix. Since the patient recovered this question must remain unsettled. The occurrence of nephritis is of interest because of its presence in a case reported by Roedelius and Brack¹¹ in 1926. Their case was one of leptothrix mycosis of the tongue. The tongue became swollen and a small area of gangrene appeared. Incision for drainage was employed but no pus was found; a piece of tissue was excised for section and culture. Following operation a leptothrix stomatitis appeared, and later an acute glomerulonephritis was noted. Sections showed

many filamentous organisms in the tissue. Attempts to culture the organism failed.

In 1921 von Meyenburg¹² saw a case in which an extensive ulcerative process in the stomach was associated with the presence of many small abscesses in the liver. Sections of the stomach showed filamentous organisms in the mucosa and submucosa. The liver showed two apparently different processes, small abscesses with central masses of streptococci, and small areas of necrosis with secondary leukocytic invasion and a few scattered cocci. A few filaments were seen in the latter lesions, but not in the abscesses. In one section masses of leptothrices were seen loosely filling two branches of the portal vein. Cultures of the abscesses contained streptococci only.

Shortly thereafter Lang¹³ reported a case of liver infarction associated with a chronic duodenal ulcer. There were many areas varying in size, club-shaped or rounded and irregular, of gray-green color, visible through the capsule and for the most part sharply circumscribed. Well defined zones of leukocytic infiltration about these areas were not seen, although a few foci of leukocytic infiltration were found. Within the bile passages at the edges of the affected regions were accumulations of long unbranched filaments and coccoidal bodies; both stained deeply with hematoxylin, but were Gram-negative. The nature of the coccoidal bodies was not clear, but they seemed to be growth forms of the leptothrix rather than staphylococci. The affected bile ducts were widened and their walls thickened. Even more massive clumps of filaments, and occasionally cocci, were seen in the portal veins, which also were for the most part greatly enlarged. The veins and some of the adjacent hepatic arteries were thrombosed, and contained masses of leptothrices. Frequently extension of filaments out into the sinusoids of adjacent and usually necrotic lobules was seen. Toxic action of the organism upon the tissues was not apparent, and definite abscess formation was lacking, the organisms acting purely mechanically by occlusion of vessels. Cultures of the organism were not obtained.

Galli-Valerio^{14, 15} has repeatedly been able to isolate a leptothrix from cases of pleuritis previously diagnosed as being of actinomycotic origin. The organism in all instances appeared to be identical with a leptothrix isolated in 1896 by Piana and Galli-Valerio¹⁶ from a dog which died of a suppurative pleuritis. They named their organism *Leptothrix pleuritica*, but considered it identical with an-

other organism named *Discomyces pleuriticus* by Rivolta twelve years previously. In 1927,¹⁷ and again in 1930,¹⁸ Galli-Valerio and Bornand reported more cases of infection by *Leptothrix pleuriticus*. In two of their total of nine cases attempts to grow the organism succeeded.

There have been reported a number of cases of meningitis with the recovery from the spinal fluid, and in some instances from the blood also, of a Gram-negative, pleomorphic, coccobacillary organism which formed filaments varying in length. These have been considered as leptothrices by the various authors, but in discussing these cases Mackenzie¹⁹ says: "All these communications however afford no conclusive evidence that the filamentous organism belongs to the higher group of bacteria." However, in cultures of a series of twenty-five cases of meningitis caused by Gram-negative organisms other than the meningococcus, Mackenzie six times isolated an organism which he considers a true leptothrix. The organisms all belonged to one species and appeared in two phases, (a) a presporing or non-sporing phase, and (b) a sporing phase.

From this review of the literature one can see that leptothrices have been discovered in the liver in only two instances. In neither of these was it proved that a leptothrix was responsible for the associated hepatic lesions. The case to be reported therefore appears to be the first recorded instance of leptothrichal hepatic abscesses.

REPORT OF CASE

Clinical History: The patient was a girl of 11 years, native of a town in north Alabama, who while on a visit to a small town in south Tennessee began having chills about Sept. 1, 1930. During the next two weeks she had fever, and complained of weakness and pain near the right costal margin. She was at first thought to have malaria, but administration of quinine afforded no relief, and blood smears failed to show plasmodia. Typhoid was next suspected, and a Widal test is said to have been positive. The symptoms were not typical of typhoid, however, and during the latter part of September her temperature was normal in the morning with an afternoon rise to 104° F. The fever persisted, and she began having abdominal distention. This was at first intermittent, but ultimately became persistent. At the end of October abdominal paracentesis was performed and a large amount of fluid was withdrawn. The puncture wound drained for two weeks before healing. X-rays of the chest are said to have shown no abnormalities. During November her fever subsided somewhat, and she began to gain strength. She had no abdominal pain, bloody stools, diarrhea, or constipation. About December 1 she developed a cough and began expectorating large amounts of viscid yellow sputum. At the same time fever,

malaise and weakness increased, and she began losing weight. She was admitted to the Vanderbilt University Hospital Dec. 19, 1931. The past and family histories are irrelevant.

Physical Examination: The significant findings were as follows: Temperature, 102.4° F, pulse 132, respirations 36. She was emaciated (20 per cent underweight), and although she was in no apparent distress paroxysms of coughing were brought on by movement. The skin was rough and scaly, and brown in color. There was no glandular enlargement. The tongue was raw and beefy and covered by white patches, and the tonsils were enlarged and injected. There was flatness at the right lung base laterally and posteriorly, and many coarse and fine râles were heard in this area. The right cardiac border could not be made out, but the apex was pushed to the left. The heart rate was rapid, but the sounds were normal. The abdomen was distended and contained fluid. The liver extended 5 cm. below the costal margin and the spleen was easily palpated.

Laboratory Findings: Urine: Specific gravity 1.020 to 1.030, reaction acid, albumin 0, sugar 0, occasional white blood cell, no red blood cells or casts.

Blood: Red blood cells 3,050,000, white blood cells 13,600, hemoglobin 38 per cent. Differential count (supravital) showed neutrophils 75 per cent, lymphocytes 19 per cent, monocytes 6 per cent. Wassermann and Kahn negative.

Of four blood cultures two were sterile and two contaminated. Agglutination of serum for typhoid was slight twice (1:40 dilution) and negative once; negative for *Paratyphoid A* and *B*, *melitensis*, *abortus*, *tularensis* and Weil-Felix.

Stools showed no parasites or ova. Tuberculin negative with 2.5 mg.

X-ray of the chest showed an abscess at the right lung base with infiltration of the surrounding parenchyma. The cardiac shadow was enlarged to both right and left.

Subsequent Course: On the day after admission 700 cc. of clear yellow fluid with a specific gravity of 1.013 were removed from the abdomen. The cell count was 1800 erythrocytes and 5400 leukocytes; of the latter 95 per cent were lymphocytes, 4 per cent polymorphonuclear neutrophils, and 1 per cent monocytes. No organisms were seen in smears or cultures. With institution of postural drainage of the lung abscess a small amount of non-malodorous pus was obtained and the cough became less severe. Eleven days after admission dilated veins were noted over the chest. At this time an X-ray following pneumoperitoneum showed adhesions between the right diaphragm and dome of the liver. The abscess cavity was poorly visualized, and there was a small amount of fluid in the right pleural cavity. The general condition improved somewhat, although there was no gain in weight. Fluid reaccumulated in the abdomen, and at the end of the third week 1500 cc. were removed. Subsequently no more fluid collected. The temperature was always elevated and showed much irregularity; it was usually above 100° F, and varied 2 degrees or more daily. The 8.00 A.M. or noon temperature was usually the lowest, and the 8.00 P.M. or midnight temperature the highest. During the fifth and sixth weeks it showed little variation, and was frequently below 100, but it again became irregular and rose above 103° F several times thereafter. X-ray of the chest at the end of the fourth week showed only the medial wall of the abscess with no longer a definite cavity. Another chest plate taken at the end of the sixth week showed little change in the lung, but much diminution of the cardiac shadow. By this time the child felt much better, and although she still had a high fever she was discharged after seven weeks in the hospital. Six days later she was brought back to the hospital in a moribund state and died a few minutes after being put to bed.

AUTOPSY FINDINGS

Gross Description: The child was much undernourished and the layer of subcutaneous fat was very thin. The skin was dry and had a grayish sallowness which did not appear to be icteric. There were a few dilated veins over the chest anteriorly. The chest was pyramidal in shape, the lower portion flaring out markedly. A couple of needle holes were seen over the heart, the result of a postmortem pericardial tap which produced nearly 300 cc. of purulent fluid. The liver edge extended 8 cm. below the costal margin in the midclavicular line.

Peritoneal Cavity: There were approximately 200 cc. of clear, straw-colored fluid in the peritoneal cavity. The liver was greatly enlarged and its margins were smooth and rounded. In addition to extending downward lower than normal it caused some elevation of the diaphragm. The omentum was reflected up over the anterior-superior surface of the liver, being adherent to it at places; it enveloped the stomach and spleen, and was closely adherent to the latter. The liver and spleen were adherent to each other, and between them was a small amount of yellowish mucoid pus. There were many fibrinous and fibrous adhesions about this abscess and over the surfaces of the spleen and liver, holding these viscera to the diaphragm. A few fibrinous adhesions were present about the pelvic organs, but the organs themselves did not appear abnormal.

Pleural Cavities: There was no fluid in either pleural cavity. Fibrous adhesions held the right lung to the diaphragm, and fibrinous adhesions were present between both lungs and the pericardium, and the diaphragm and left lung base.

Pericardial Cavity: The pericardium was thickened, and when the pericardial sac was opened a shaggy deposit of fibrin was seen on all the pericardial surfaces with thick festoons connecting the visceral and parietal layers (Fig. 1). Approximately 100 cc. of thick, yellow, purulent fluid remained in the pericardial sac. In the anterior portion of the pericardium near the apex of the heart was a small circumscribed collection of slightly yellowish mucoid pus from which smears were made. The thoracic viscera, diaphragm and liver were removed in one piece.

Heart: A section through the ventricles revealed a very pale pink, soft myocardium. Further examination was not made as it was thought desirable to save the specimen intact.

Lungs: In general the lung tissue was normal, although there was some atelectasis. A small area of consolidation was found at the base of the right lower lobe, obviously the residuum of the lung abscess, as comparison with the roentgenograms showed. A fortunately directed section passing through the liver, diaphragm, and right lung base showed both the nature and manner of origin of the former lung abscess (Fig. 2, inset). An abscess in the upper part of the liver ruptured through the diaphragm into the pleural cavity, producing an adhesive pleuritis. Radiating from the thickened pleura into the lung was an area of fibrosis. Within the lung, pleura and diaphragm were small yellow flecks similar to those to be described in the liver.

Gastro-Intestinal Tract: Not remarkable.

Liver: When the common bile duct was cut a light yellow mucopurulent material escaped. Pressure upon the gall-bladder caused the escape of thick, dark brown bile. The gall-bladder wall was edematous. Several superficial abscesses were found in the liver by palpation, and a yellowish mucopurulent material was aspirated from one and used for cultural study. Sections through the liver revealed the presence of a great many abscesses varying in diameter from a few millimeters to 3 cm. These abscesses were grouped irregularly in the upper portion of the liver, and were abundant in both right and left lobes. A peculiarity of their distribution is that although the abscesses were very numerous in the lateral half of the right lobe and extended down quite far toward the inferior margin, in the medial half of this lobe they were much less numerous and were found only in the upper portion. There was, however, a continuous chain of abscesses extending from the outermost edge of the right lobe to the outermost edge of the left lobe. All these abscesses appeared to be in association with bile ducts and contained a light yellow, or grayish mucoid pus. The pus was perfectly smooth and contained no granules. When the pus was permitted to escape from them the majority of the abscesses were seen to have irregular walls, while the larger spaces had smooth, glistening walls and were obviously greatly dilated bile ducts. The walls of both abscesses and bile ducts were stained dark green by bile. The abscesses and, to a less marked degree, the dilated, pus-filled bile ducts were bordered by an irregular zone of bright yellow or orange tissue (Figs. 1 and 2). A section passing through the left lobe of the liver, diaphragm and

pericardium near the apex of the heart showed a large abscess between the pericardium and liver capsule, the diaphragm in this area having become necrotic and disappeared (Fig. 1). The liver capsule was much thickened. Within the abscess were many shaggy bands of fibrin surrounded by a thick, white, mucoid pus. Numerous abscesses were present in the upper part of the left lobe, and a communication could be demonstrated between one of these liver abscesses and the diaphragmatic abscess. The pericardium about this abscess was thickened in some places and quite thin in others. Although no fistulous connection between the pericardial cavity and the diaphragmatic abscess was found, it was obvious that the infection extended from the abscess through the pericardial sac to the pericardial surfaces. Although the sinusoids were congested, the liver tissue surrounding the abscesses was pale and whitish pink in color.

Spleen: The spleen weighed 270 gm. It was deep reddish purple in color and contained numerous, small, blackish areas of hemorrhage. There was some increase in connective tissue. No abscesses were seen.

Kidneys: The kidneys weighed 160 gm. each. They were enlarged and soft. The cut surface bulged outward markedly and showed extreme congestion. The ureters and bladder were normal.

The pancreas, adrenals and genital organs were not remarkable.

MICROSCOPIC EXAMINATION

Heart: The surfaces of the visceral and parietal pericardium are greatly thickened by a newly formed fibrous tissue with many fibroblasts and delicate collagen fibrils and numerous strands of unorganized fibrin. This tissue is very vascular and is infiltrated by moderate numbers of polymorphonuclear leukocytes, plasma cells, and a few large mononuclear leukocytes. Areas of hemorrhage are numerous in the parietal pericardium. The serosa is completely missing. Upon the superficial layer of the pericardium there is a thick, irregular layer of fibrin and many polymorphonuclear leukocytes (Fig. 5). A section taken through the abscess near the apex of the heart shows a much more chronic process than is seen elsewhere in the pericardium. The connective tissue is very dense and is infiltrated by large numbers of plasma cells, polymorphonuclear

neutrophils, and large mononuclear leukocytes. The diaphragm is also infiltrated by these cells. The muscle cells are atrophic and many of them have disappeared. The mononuclear leukocytes are greatly enlarged and have a foamy cytoplasm; they are abundant, and in many areas are the predominant cells. There are many foci in which tissue liquefaction has occurred, and about the margins of these foci is a zone of large mononuclear leukocytic infiltration.

Lungs: The sections show some collapse of the alveoli, with moderate numbers of polymorphonuclear leukocytes within the alveolar capillaries. A section from the left lower lobe includes the parietal pericardium. Between the visceral and parietal pleura is a small amount of fibrinous exudate containing a few polymorphonuclear leukocytes. The serosal cells are well preserved, and there is no organization of the exudate. Beneath the parietal pleura is the much thickened parietal pericardium which has already been described. Duplicates of this section stained by Goodpasture's carbol anilin fuchsin stain²⁰ show moderate numbers of leptothrices within the pericardial exudate (Fig. 7). Sections taken from the area of consolidation in the right lower lobe show great thickening of the alveolar walls, which in part results from fibrous tissue proliferation radiating outward from some of the bronchioles. Numerous foci of lymphocytic infiltration are seen. Some alveoli are completely effaced, but many are still patent, although nearly completely collapsed. These alveoli are lined by large cuboidal epithelial cells, and some contain a few polymorphonuclear leukocytes or a large mononuclear phagocyte with foamy cytoplasm. Some bronchioles contain many polymorphonuclear leukocytes and some desquamated epithelial cells. The pleura is much thickened by fibrous tissue proliferation and is adherent to the diaphragm, and within the pleura, diaphragm and bit of included liver are many groups of large, fat-laden mononuclear phagocytes identical with the occasional cell seen in the alveoli. These obviously gave rise to the yellow flecks seen in the gross. Duplicates stained by the carbol anilin fuchsin stain show many leptothrices within the purulent exudate in the bronchioles (Fig. 6), and some are seen within polymorphonuclear leukocytes. An occasional leptothrix is seen within an alveolus, but none is seen within the lung tissue or scar tissue.

Liver: In the region of the abscesses the liver capsule shows irregular areas of slight thickening in which are some new blood channels,

but neither fibrin nor leukocytic infiltration. The sinusoids are congested and many contain small thrombi of hyalinized fibrin. The cells are atrophic, particularly those about the central veins and adjacent to the abscesses and areas of fibrosis, and many cells contain small fat vacuoles. In some areas hemorrhage has occurred into the cords of cells, and in others necrosis of the cells is seen. The bile ducts appear to be increased in number, and there is a great increase in fibrous tissue about them. Polymorphonuclear leukocytes are seen within some of the ducts and in the surrounding connective tissue. This increase in connective tissue is marked in some areas, many whole lobules being entirely replaced, although in some instances a few liver cells persist. This tissue is infiltrated by large numbers of plasma cells, lymphocytes, neutrophilic and eosinophilic polymorphonuclear leukocytes, and large mononuclear leukocytes, and represents an effort at localization of the abscesses (Fig. 3). Many abscesses of uneven size and irregular outline are seen. The material within their centers consists mainly of polymorphonuclear neutrophils, but at their margins where tissue liquefaction has not yet occurred the other leukocytes just mentioned are abundant. The tissue at the periphery of these abscesses is infiltrated by large numbers of fat-laden, large mononuclear leukocytes (Fig. 4), many of which contain masses of pigment varying in color from dull yellow to orange, and some containing hemosiderin or hematoidin. The abscesses are obviously of various ages, some are very young, and others are healing. Sections stained by Goodpasture's carbol anilin fuchsin stain show moderate numbers of long bacillary forms and some filamentous organisms in the abscesses.

Spleen: There is a diffuse increase in the fibrous tissue of the spleen.

The gastro-intestinal tract, pancreas, adrenals, kidneys and bladder show no significant changes.

Comment: The essential features of this case consist of the presence of multiple liver abscesses, a diaphragmatic abscess and a subacute pericarditis, all demonstrated to be produced by a leptothrix, and a lung abscess unquestionably produced by the same organism. Rupture of a liver abscess led to formation of a subdiaphragmatic abscess with subsequent necrosis and disappearance of all the diaphragm in a rather large area. Although no actual communication between the diaphragmatic abscess and the pericardial cavity was

found, there can be no question that the pericarditis arose as a result of direct extension of the infection from the diaphragmatic abscess. Communication between the former lung abscess and a liver abscess was demonstrated, and although it is possible that the lung abscess was the primary focus of infection, it seems much more likely that it occurred as a result of rupture of a liver abscess. The source of the organism and the portal of entry to the body are obscure. No lesion of the gastro-intestinal tract was found which might have permitted invasion by the organism; however, there may have been such a lesion which had healed and left little or no trace of its presence.

BACTERIOLOGY

Smears: Smears of pus from the pericardial and liver abscesses showed many leukocytes and a few delicate Gram-negative filaments which showed no branching and varied from 20 to 150 microns in length, the majority measuring around 100 microns. Many of these filaments were beaded and a few were relatively thick, tapering off at each end. A few examples of phagocytosis of these filaments by polymorphonuclear leukocytes were seen. No other microorganisms were present.

Cultures: Aerobic cultures were made of the pus obtained from the liver abscess, the following media being used: yeast broth, ascitic fluid, glucose broth-ascitic fluid, blood agar plate, Sabouraud's plate, Sabouraud's stab and agar stab. An anaerobic culture with yeast broth and $\frac{1}{8}$ cc. of pus was also made. Incubation was carried out at 36° C unless otherwise noted. The glucose broth-ascitic fluid tubes received a heavy inoculation, and a few organisms were later found in smears from one of the two tubes, a small amount of growth presumably having occurred. After 3 days incubation a small amount of growth was noticed in the anaerobic tube. After a couple of days the growth was more abundant, but heavy growth never occurred. Smears showed long filaments identical with those seen in the smears of pus. Anaerobic subcultures of this tube were made using yeast broth and yeast broth plus ascitic fluid with vaseline seal, and aerobic cultures using blood agar plates, Sabouraud's medium, ascitic fluid with glucose, litmus milk, potato, and potato in yeast broth. Growth occurred in none, and anaerobic subcultures were again tried, using yeast broth with vaseline seal to which a small

piece of fresh kidney had been added. Growth occurred in this medium fairly well, the organism appearing as a flocculent mass at the bottom of the tubes. The sediment from two of these tubes was inoculated subcutaneously into each of two guinea pigs (Nos. 3 and 4) with results to be discussed more fully later; suffice it to say at present that abscesses developed and that pus was taken from one of these (Guinea pig 3) and cultured anaerobically with recovery of the organism.

The original culture was carried through 5 generations and the strain was then discarded, since a contaminating organism had been introduced by the fresh tissues used. A repetition of this accident was avoided by heating the tubes to 60° C for 30 or 45 minutes immediately after addition of the tissue. This heating was also intended to stop enzymotic action. Liver was at first used as well as kidney, but its use was not continued as fresh liver is frequently not sterile, and the introduction of contaminated tissue was avoided as much as possible. Subsequently only kidney was used. This medium permitted fairly free growth of the organism, but was rather unsatisfactory because of the precipitate formed as a result of heating it to ensure sterility; this precipitate was rather abundant and concealed the amount of growth of the leptothrix. Subcultures of the original strain, which we shall call Strain 1 for convenience, were made using five blood agar plates, two Sabouraud's medium plates, two flasks of bread medium (toasted bread crumbled with enough saline added to make a firm gell after sterilization) and one glucose agar plate containing ground up liver, all kept in an atmosphere of carbon dioxide. No growth occurred in any of these. One Sabouraud's plate in CO₂ was kept at room temperature but showed no growth. Two Sabouraud's plates and two blood agar plates were incubated in a chamber which had been exhausted to a pressure of 25 mm. of Hg and in which the remaining oxygen was removed by the use of pyrogallol; no growth occurred here either. A third flask of bread medium was incubated aerobically, but no growth occurred. All these media were heavily inoculated, the inoculum being obtained from tubes of liver or kidney broth in which the strain was maintained. This strain was still growing vigorously at the time it was discarded, the contaminant which it had acquired apparently having no effect on its growth. Inasmuch as the organism had been meanwhile obtained in pure culture again and was growing vigor-

ously, no advantage was seen in maintaining the contaminated strain further. All but one of the subsequent cultures were anaerobic.

Pus from the abscess in Guinea pig 3 was inoculated into two tubes of broth, three tubes of liver broth, and two tubes of kidney broth. The plain broth tubes received the heaviest inoculation, and after 48 hours many small round colonies were seen on the side of one tube (Fig. 8); the other tube contained only two colonies, but these grew much larger than those in the first tube. Only one of the five tubes containing tissue showed growth, but when the others were reinoculated with colonies from the plain broth tubes abundant growth occurred. The strain so established we shall call Strain 2. This strain was carried through 16 generations and furnished material for most of the animal inoculations. In addition to the various culture media which have been previously mentioned as having been used, the following were tried with this strain of the organism: Dorsett's egg medium (aerobic and in CO₂), yeast broth with egg yolk, and glucose broth with egg yolk; the effect of addition of glucose to the broth and tissue was also tried. No growth was obtained on Dorsett's medium. The second egg medium gave good growth if pus and glucose were present, but it was unsatisfactory because of the sediment in it. Glucose seemed to retard rather than to augment growth except in the egg yolk medium, growth not occurring in its absence in this medium.

A third strain was recovered from a subcutaneous abscess produced in Guinea pig 7 by Strain 2. This strain was carried through 5 generations.

Characteristics of the Organism

A. Morphological: The organism seen in smears of the cultures was unquestionably the same as the one seen in smears of the pus. It was Gram-negative, and stained poorly with most stains. Dilute carbol fuchsin gave the best results, although Wright's stain and Loeffler's methylene blue gave good results when prolonged staining was employed. Many filaments were beaded, but none appeared to be formed of strings of bacilli. Wright's stain brought out the beading extremely well, but it was still quite conspicuous in carbol fuchsin and methylene blue stains. The filaments did not bend sharply, but formed smooth sinuous curves. No clubbing of the ends of the filaments was noted, but some very thick fusiform filaments

were seen (Fig. 14). In one smear stained by Wright's stain, what were at first thought to be spores were seen (Fig. 10); spore stains on other smears from this same culture showed that these structures were not spores — they appeared to arise as a result of longitudinal splitting of the filaments. Similar structures were not seen subsequently, and never again was anything suggestive of a spore seen. The filaments varied in length; some cultures showed many bacillary forms (Fig. 12) and others showed very long filaments (Figs. 9 and 13); many filaments were seen exceeding 300 microns in length. The bacillary forms were most numerous in media which offered less favorable conditions for growth. In no instance was unquestioned branching observed, although some instances were seen in which a short filament lay at right angles to another filament with its end touching the side of the other (Fig. 10). There is no doubt that the majority of these were only fortuitous. Where flocculent growth of organisms occurred they were pretty much matted together, and the colonies consisted of matted masses forming tough clumps which were mashed or teased only with some difficulty (Fig. 9).

B. Cultural: The best growth was obtained in yeast broth containing a small piece of fresh kidney; whether heating made this medium better or not is not known. A fairly good medium was found to be yeast broth to which sterile pus had been added, or pus containing the leptothrices. Some growth occurred under partial aerobic conditions, as evidenced in the glucose-broth ascitic fluid inoculated with pus, and also by a tube of broth containing kidney which was inoculated and not resealed by vaseline. In these tubes bacillary forms of the organism predominated, but they were also numerous in the egg yolk-broth medium, and occasionally were very abundant in the broth-kidney medium. In the tubes of broth containing pus, discoid colonies were formed on the sides of the tubes. The colonies showed some variation in size, but averaged about $\frac{2}{3}$ mm. in diameter. Their centers were more transparent than their edges (Fig. 11), and radiating outward from their edges was a feathery tangle of filaments (Fig. 9). Generally growth occurred with colony formation on the tissue and on the sides of the tubes. The latter colonies were always small and were easily dislodged, many falling to the bottom of the tube at the slightest jar. The colonies growing on the tissue were not discoid in shape but grew out equally in all directions, forming hemispheres. They were somewhat

less tough than the colonies formed in the pus broth tubes, and they reached a considerably larger size, one of them reaching 5 mm. in diameter. The colonies in the tubes grew to larger sizes when only a few colonies were present. When subcultures were made, one of the colonies was fished out with a loop and rubbed on the side of the tube to be inoculated in order to break up the colony as much as possible. Since some colonies were more easily broken up than others, there was much variation as to the amount of breaking up that occurred. In some tubes colony growth did not occur so readily, a more flocculent type of growth being found. It seems possible that this may have resulted from more thorough breaking up of the inoculum, although it is probable that many colonies were formed in these tubes and concealed by the precipitate within them. Subcultures of a culture kept at room temperature for 1 month failed to grow. Subcultures of a culture refrigerated 6 months showed no growth.

Summary of Cultural Characteristics: Fairly free growth occurred only under "anaerobic" conditions in yeast broth containing pus, or fresh or heated animal tissue. Growth was not obtained on solid media. Occasionally flocculent growth occurred, but usually definite colonies were formed. Under less favorable conditions bacillary forms were abundant.

Animal Inoculation

One-half cc. of pus from the liver abscess was given to each of two rabbits, two guinea pigs, and two baby rats. One rat received intraperitoneal, and the other intrapleural injection, but showed nothing. The guinea pigs were inoculated subcutaneously; one pig showed only some induration about the site of inoculation for several days, but the other developed a small firm papilla which began to recede after 9 days, and 4 days later had disappeared; 3 days still later all induration had disappeared. One rabbit received the pus intravenously, and the other received it intraperitoneally, an attempt being made to puncture the liver and inject the pus into it. The first rabbit showed no fever at any time. Three weeks after inoculation it was killed, but autopsy revealed nothing of interest. The second rabbit was killed after a month but nothing of interest was seen.

Meanwhile the organism had been found in the anaerobic culture of the pus and had been transferred to several tubes of broth-kidney medium. After several days growth the tissue was removed from two tubes and the tubes centrifuged; most of the supernatant fluid was poured off and the sediment used for inoculation. One guinea pig (No. 3) was inoculated subcutaneously with the material from a 7 day culture, and developed a large firm nodule at the site of injection. Sixteen days later it was killed and nothing of note was found except an encapsulated abscess at the site of inoculation. This contained a yellowish white pus; smears contained leptothrices, and anaerobic cultures contained many colonies (establishing Strain 2). The second tube, an 11 day old culture, was injected subcutaneously into another guinea pig (No. 4), two sites of inoculation being chosen, and two nodules appearing. After 3 days the nodules diminished in size, and after 2 weeks had disappeared. At the same time two baby rats and one mouse were given some of the culture subcutaneously, but showed no reaction. Subsequently three more guinea pigs received cultures subcutaneously. Nodules formed as before, and after 16 days pus was aspirated and cultured from one pig, establishing Strain 3. The nodules in all three gradually diminished and disappeared after about a month. A rabbit was inoculated subcutaneously with material from two cultures. Fourteen hours after inoculation a large vesicle appeared but rapidly receded; underneath it however was an area of induration which gradually developed into a nodule nearly 1 cm. in diameter. Seventeen days later aspiration was attempted, but failed because of thickness of the contents. Thirty-eight days after inoculation the abscess ruptured spontaneously.

Intraperitoneal inoculations with cultures were tried with three baby rats and two guinea pigs. The rats died 2 days later and showed nothing. The guinea pigs appeared very ill for a few days, but rapidly recovered and were apparently normal a week later. One of the two pigs was killed 12 days after inoculation. A small globule of pus was found free in the peritoneal cavity, and another was found attached to the wall of the seminal vesicle. A culture of the pus produced many colonies. This strain was subcultured but failed to grow. It was thought that if a sterile peritonitis could be produced it might be possible to establish the leptothrix within the peritoneum. Accordingly a guinea pig was given some aleuronat in-

traperitoneally shortly before the culture was given. The animal became very ill, but soon recovered. A mass was palpable in the abdomen, and after 25 days the pig was killed. Several loops of small intestine and the seminal vesicles were adherent to each other and to the anterior abdominal wall. A small yellow spot was found on one seminal vesicle. This was cultured, but no growth occurred.

Intravenous injection of cultures was tried with two rabbits. The first received all the material in two 4 day old cultures and had a diarrhea for a week, but never developed fever. Eighty-one days later the animal was killed, but the tissues showed nothing of note. The second rabbit received a single colony which measured 5 mm. in diameter, the remainder of the material in the tube being rejected. This animal showed no reaction of any kind.

Intrahepatic injection of material from three cultures was carried out on one rabbit. The abdominal cavity was opened and the liver injected in two places. Some leakage was seen to occur at the sites of injection. One month later the animal was killed, but no definite lesions were found.

An attempt to enhance the virulence of the organism by rapid passage from animal to animal met with early defeat. Subcutaneous inoculation of one guinea pig with pus removed from an abscess produced by injection of a culture of the organism in another pig resulted in the formation of a small nodule which very soon disappeared.

As controls each of two guinea pigs was injected subcutaneously with the sediment in two tubes of unused sterile broth-kidney medium. One showed no reaction, and the other developed a small nodule which very soon disappeared.

Summary of Results of Animal Inoculation: Abscesses were produced in guinea pigs and rabbits by subcutaneous inoculation of cultures. Intravenous and intraperitoneal injection of cultures caused only transitory indisposition of the animals.

DISCUSSION

The amount of experimental work done with this organism is admittedly inadequate. More extensive cultural studies including fermentation reactions with sugars were planned, but could not be done because of the special growth requirements of the organism.

An attempt to increase virulence by rapid passage from animal to animal was abandoned when it was found that the number of organisms in the abscesses formed were not adequate to produce anything other than a very slight reaction in a second animal. Direct inoculation into the liver gave equivocal results and was not repeated. Peritonitis could not be produced, although subcutaneous inoculations were successful. The results obtained are not without value however, and may offer help whenever a similar organism is recovered in the future. The organism recovered from this case is obviously different from all pathogenic leptothrices which have been heretofore described, and there is no evidence to indicate that any two of the leptothrices previously reported to be pathogenic to man are the same. The leptothrix producing the disease in this case seems to have a higher pathogenicity for man than those previously encountered, as evidenced by the intense reaction to the presence of relatively few organisms. The fatal outcome of the case is another evidence of high pathogenicity, although the presence of the severe pericarditis makes it impossible to say whether or not the child would have died because of the liver abscesses. It is quite possible that she would not, for many liver abscesses were healing and the lung abscess healed nearly completely.

SUMMARY

1. A summary of the literature pertaining to leptothrices pathogenic to man is presented.

2. Report is made of a case of leptothricosis in which multiple liver abscesses were formed, with rupture of one abscess into the base of the right lung and formation of a lung abscess, and rupture of another in the left lobe of the liver, leading to the production of pericarditis.

3. The organism was isolated in pure culture from the liver and successfully carried *in vitro* through many generations. No other organisms were present in the lesions.

4. Experimental work with the organism showed it to be slightly pathogenic for rabbits and guinea pigs.

5. This organism has apparently never been previously encountered.

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DESCRIPTION OF PLATES

PLATE 12

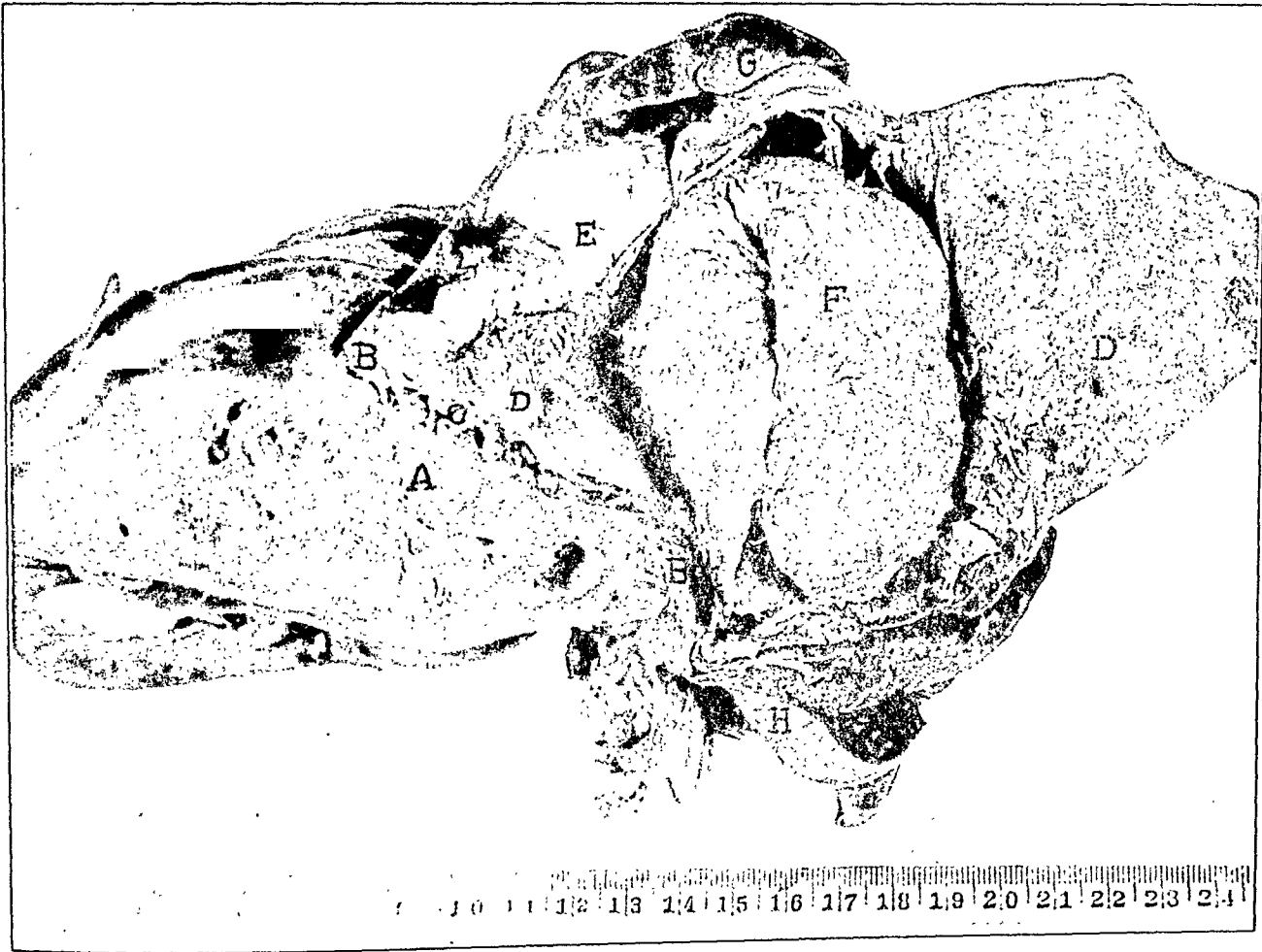
FIG. 1. Thoracic viscera and liver. The normal relations have been somewhat distorted in fixation.

A = cut surface of left lobe of liver showing multiple abscesses. B = diaphragm. C = abscess which has destroyed a portion of the diaphragm. D = inner surface of the parietal pericardium. E = outer surface of pericardium. F = heart. G = right lung. H = left lung.

FIG. 2. Right lobe of liver and right lung base.

The large photograph shows the numerous abscesses, dilated bile ducts, and the yellow and orange flecks in the liver. Infiltration of the lung base is also seen.

The inset shows the reverse surface of this slab, about 1 cm. lateral to the other surface. Note rupture of abscess through the diaphragm, adhesive pleuritis and extension of infection into lung.



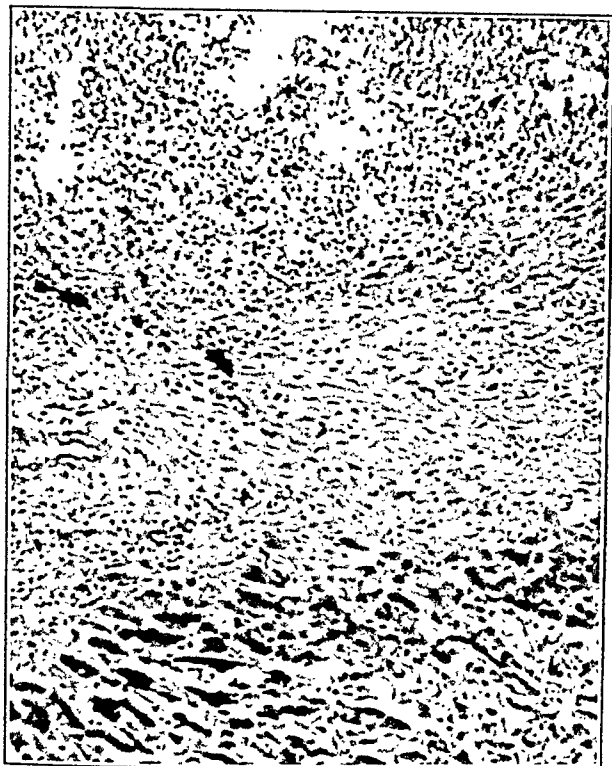
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PLATE 13

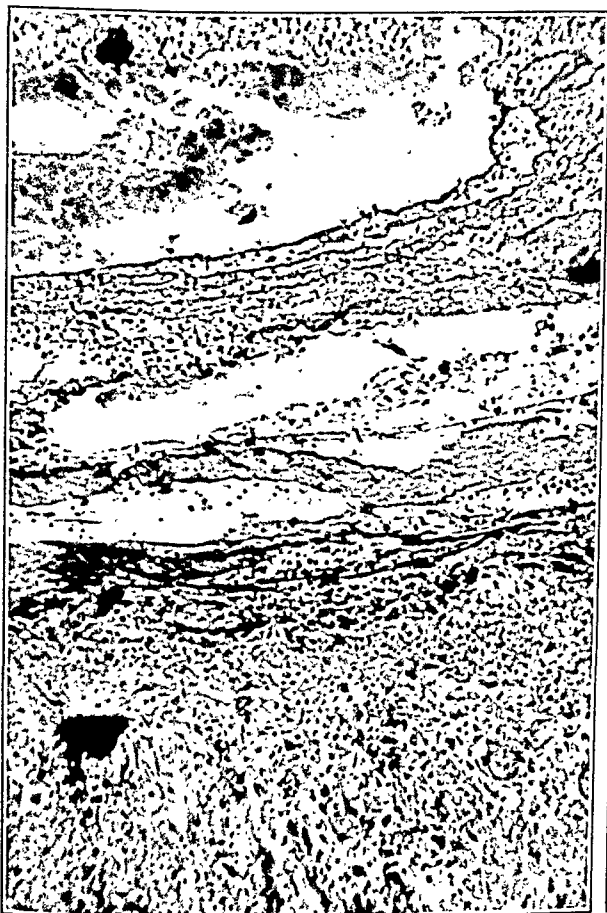
- FIG. 3. Liver. Abscess surrounded by large amount of fibrous tissue. Hematoxylin and eosin stain. $\times 80$.
- FIG. 4. Liver. Edge of large abscess with numerous fat-laden monocytes at outermost border. Hematoxylin and eosin. $\times 80$.
- FIG. 5. Pericardium. Serofibrinopurulent exudate into pericardial cavity above, granulation tissue of thickened pericardium below. Hematoxylin and eosin. $\times 80$.
- FIG. 6. Leptothrices in exudate in bronchus at right lung base. Carbol anilin fuchsin stain. $\times 2000$.
- FIG. 7. Leptothrix in pericardial exudate. Carbol anilin fuchsin stain. $\times 2300$.



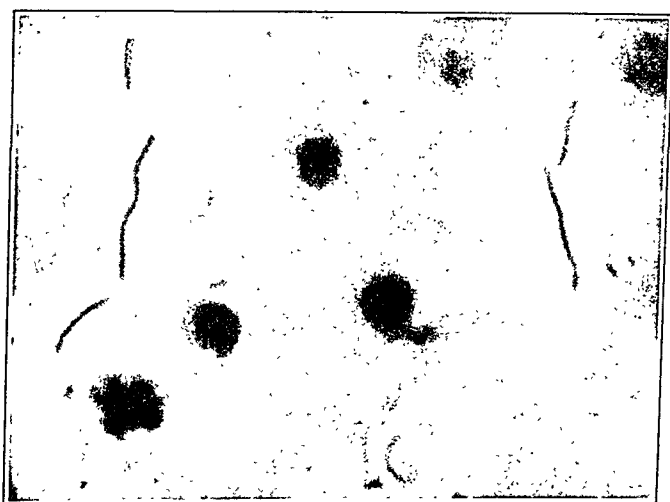
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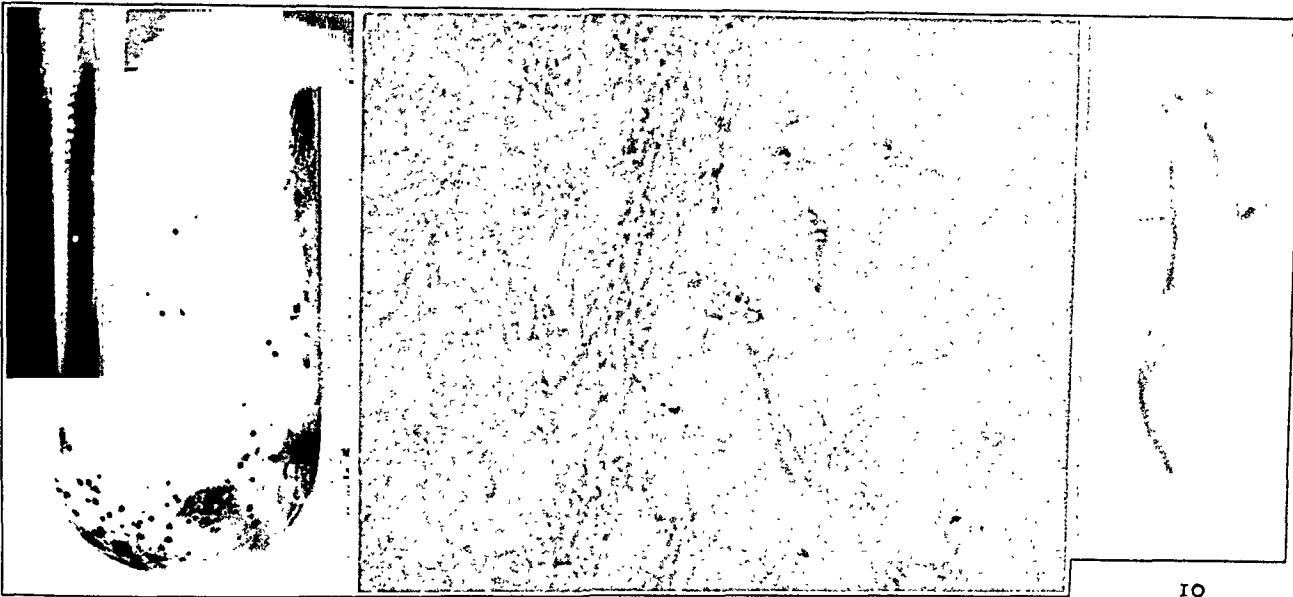
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Harris

Multiple Liver Abscesses Caused by a Leptothrix

PLATE 14

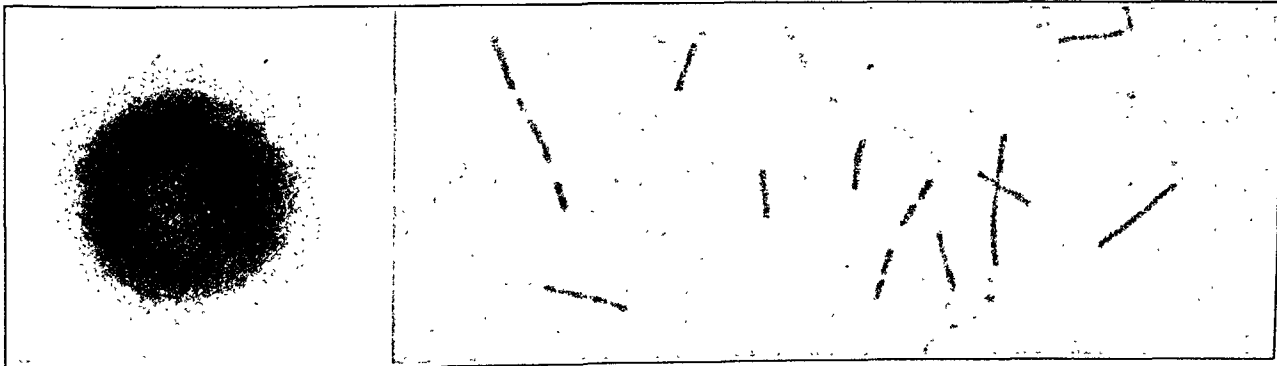
- FIG. 8. Test tube containing numerous colonies of leptothrices. Culture of pus from subcutaneous abscess of guinea pig. $\times 1.8$.
- FIG. 9. Edge of one of the colonies in Fig. 8 illustrating the tangle of filaments with outward radiation of single filaments. Unstained. $\times 800$.
- FIG. 10. Smear of culture showing structure at first thought to be a spore. This organism also shows one of the very rare examples of what may be branching. Wright's stain. $\times 2300$.
- FIG. 11. Colony from Fig. 8 showing pale center and radiation of filaments out from the edge. Unstained. $\times 120$.
- FIG. 12. Smear of culture showing bacillary forms. Dilute carbol fuchsin. $\times 2300$.
- FIG. 13. Smear of culture showing beading of filaments, and variation in thickness and density of staining of various filaments. Dilute carbol fuchsin. $\times 2300$.
- FIG. 14. Smear of culture showing type of thick fusiform structure occasionally seen. Observe the tangled mass of long filaments and beading of some filaments. Dilute carbol fuchsin. $\times 2300$.



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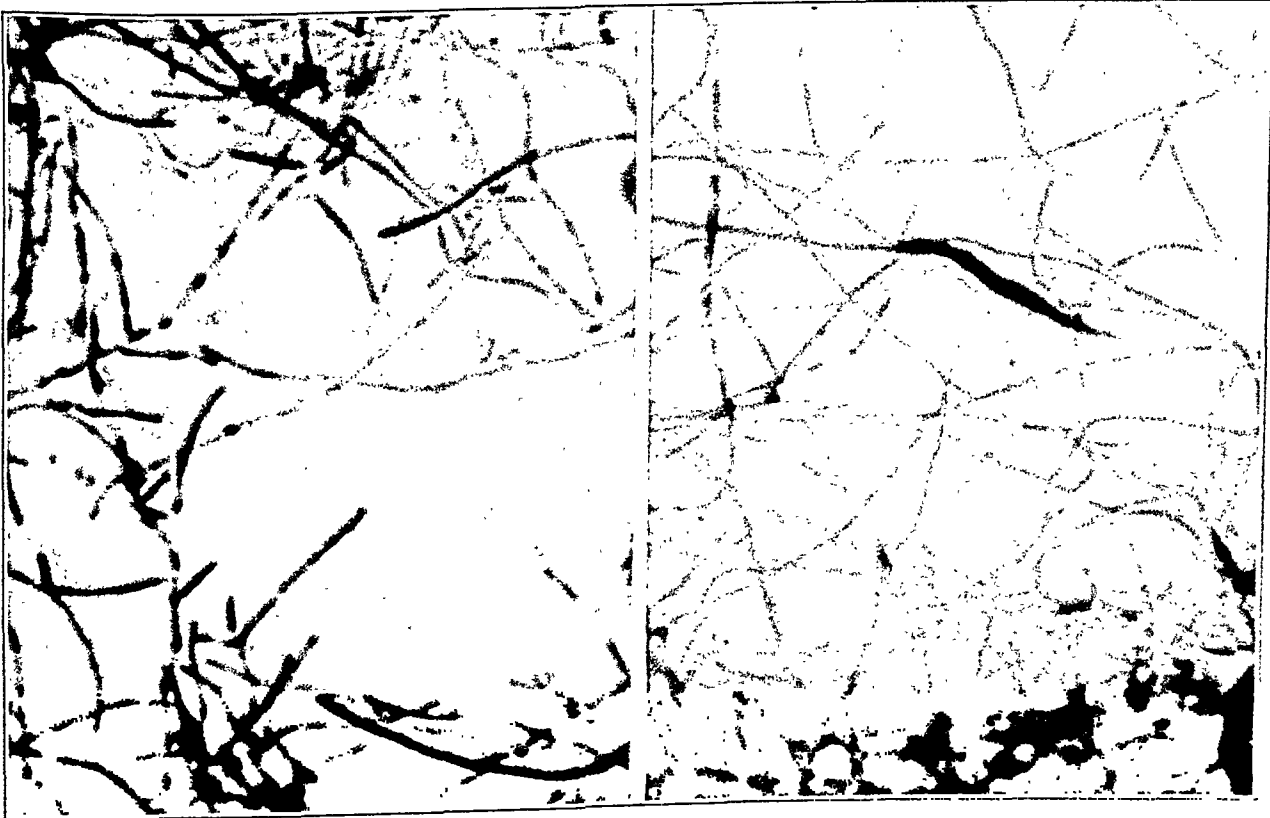
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HISTOLOGICAL STUDY OF A CASE OF THE EASTERN TYPE OF ROCKY MOUNTAIN SPOTTED FEVER *

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Although the disease now known as Rocky Mountain spotted fever had undoubtedly existed in Montana and Idaho for years, it began to receive attention only forty years ago, and it was not until 1896 that it was first described as a clinical entity.¹ Cases of the disease were soon recognized in other western states. The first case seen east of the Rockies was reported by LaBier in 1925.² The patient was a child, native of Terre Haute, Indiana, who was bitten by a "bug" which, although not identified, was presumably a tick. The child developed a disease clinically similar to Rocky Mountain spotted fever and eventually recovered. The Weil-Felix reaction was not determined. It remained, however, for Dyer, Rumreich and Badger^{3, 4, 5} in 1931 to establish upon an immunological and clinical basis beyond all doubt the existence of Rocky Mountain spotted fever in the eastern United States. In 1926 Maxcy⁶ demonstrated the existence of endemic typhus in the southeastern states, and since the publication of his studies cases of spotted fever have doubtless been erroneously regarded as typhus.

In an analysis of 100 selected cases separated on the basis of epidemiological considerations into two groups of equal size, the essential differences between the two diseases as enumerated by Dyer and his coworkers^{4, 5} are as follows: Cases of endemic typhus were predominantly of urban origin and occurred in association with rat infestation. Rocky Mountain spotted fever occurred in persons living in rural sections, or having rural contact, on premises as a rule rodent-free, and in a significantly high percentage of cases followed shortly on tick bite. As well as could be estimated, the incubation period of typhus was seven to fourteen days, while that of spotted fever was usually less than a week. The rash in typhus appeared first on the chest and abdomen, over the legs anteriorly and laterally, and frequently on the medial surface of the upper arms. In many

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cases there was no further extension, in a rather large number the back was next involved, and in the more severe cases the eruption became generalized. The face was always free, and in only one case of their series were the palms and soles involved. The rash was macular, rose or dull red in color and faded on pressure. In some cases many lesions were papular. The rash persisted for two to nine days and then rapidly disappeared. In spotted fever the rash nearly always was first seen on the wrists and ankles; it appeared next on the back, and rapidly became generalized. The palms and soles were frequently, and the face occasionally, involved. The lesions were at first roseolous macules, but by the middle of the second week they had become petechial in all but the mildest cases. At this stage and subsequently the rash was purpuric and as a rule most abundant on the wrists and ankles, legs, upper part of the back, shoulders, lateral surfaces of the arms, chest, abdomen, palms and soles, and face — in the order mentioned. Petechiae, when numerous, often became confluent. Evidence of the rash sometimes persisted for several weeks as dusky purplish or yellowish brown spots. In 36 per cent of the spotted fever cases the spleen was enlarged, and was usually tender; it was palpable in two and tender in one of the typhus group. Rigidity of the neck with presence of Kernig's sign was noted in many of the spotted fever cases, but in only one case of typhus. A small ulcer at the site of the tick bite, with enlargement of the regional lymph glands, was occasionally noted in spotted fever. Disturbances of the central nervous system were much more severe in spotted fever. In both diseases lethargy, often associated or alternating with insomnia, restlessness or irritability, was present in nearly all cases. Stupor was more frequent and coma was present, only in spotted fever. Meningismus was frequent in spotted fever and sometimes there was violent delirium. In endemic typhus the leukocyte count was generally normal or moderately diminished, while definite leukocytosis was present in spotted fever. In the endemic typhus series there were no secondary cases in any family. In the Rocky Mountain spotted fever type group there were three instances of occurrence of multiple cases in a household. In one instance three cases occurred at intervals of twelve days and seven weeks. In each of the other two there were two cases which occurred within a week of each other. Cases of typhus were rare among children, but spotted fever cases among children were more numerous. No deaths oc-

curred in the typhus group, while seven deaths occurred in the spotted fever group. Of 93 cases of spotted fever occurring in five states and the District of Columbia during the spring and summer of 1930 death occurred in twenty-one instances (23 per cent).

Until the appearance of Wolbach's studies on Rocky Mountain spotted fever⁷ knowledge of the pathology of the disease was very inadequate. Wolbach collected reports of fifteen autopsies on spotted fever cases and added five more. In approximately ten of these twenty cases the central nervous system was examined and found to be free of lesions. Because of this the presence of brain lesions has been considered as of great diagnostic value in differentiating typhus from spotted fever. This criterion has recently been shown to be inaccurate in so far as the Eastern form of spotted fever is concerned. Dyer, Rumreich and Badger^{3, 5} found brain lesions in approximately half of their guinea pigs and in two monkeys which had been inoculated with blood from cases of the Eastern form of Rocky Mountain spotted fever. Shortly thereafter Lillie⁸ was able to demonstrate lesions in the brains of five people who died of a disease identified by Rumreich as being clinically and immunologically Rocky Mountain spotted fever. Lillie gives a full account of the pathology of these cases and compares it with the pathology of the Western form of spotted fever and with that of European typhus. The lesions observed in our case are nearly identical with those described by Lillie.

REPORT OF CASE

Clinical History: The patient was a white girl of 5 years, native of a small town in west central Tennessee, who was admitted to the Vanderbilt University Hospital June 4, 1931 for diagnosis.

Seven days before admission she developed fever, was sluggish and sleepy, and failed to get up out of bed in the morning. She remained in bed thereafter, had a temperature of 102 to 103° F, was drowsy or even stuporous during the day, and at night was restless and irritable. She occasionally complained of "stomach-ache," but only once complained of headache. Two days after the onset of illness an erythematous rash was noticed over the backs of the hands and lower legs. The rash rapidly became more extensive and soon spread to the body. During the last three days before admission she cried when moved, and for twenty-four hours her neck seemed stiff. Subsequently it was discovered that a tick had been removed from her shoulder about a week before she became ill.

Physical Examination: Temperature 105, pulse 152, respirations 36. The child was drowsy but rational, and much dehydrated. She was at times restless, and any handling seemed to hurt her. The skin over the arms, legs and trunk

was covered by a rash which in places was macular and faded somewhat on pressure, and in other places was petechial. The neck was slightly stiff, and Kernig's and Brudzinski's signs were present. The heart, lungs and abdomen were not remarkable.

Laboratory Findings: Urine: amber, acid, specific gravity 1.015, albumin 0, sugar 0. There was an occasional white blood cell; no red blood cells or casts were present.

The blood showed white blood cells 15,600. Hemoglobin 12.6 gm. Differential count: polymorphonuclear leukocytes 79 per cent, lymphocytes 20 per cent, monocytes 1 per cent. Wassermann and Kahn negative. Blood culture negative.

Subsequent Course: Lumbar puncture produced a normal fluid under slightly increased pressure. On the following day the rash had spread, and there were bright petechiae covering the chest, neck, face and arms. The face, hands and feet appeared swollen. She was stuporous and at times irrational. A second lumbar puncture revealed increased sugar but nothing else. The temperature meanwhile had varied between 100 and 104° F, but was usually around 102 or 103° F. Application of a tourniquet to the arm on the third day in the hospital caused the appearance of more petechiae. A stool contained changed blood. By this time the petechiae had darkened, some were confluent, and numerous bluish blotches appeared on the body. The edema became more marked and the eyes were swollen shut. Albumin appeared in the urine on the fourth day in the hospital. Weil-Felix reaction on blood drawn on the second day in the hospital was negative, but blood drawn three days later gave a positive reaction in a dilution of 1:40. Her condition failed to improve, and on the sixth day in the hospital the temperature rose to 105° F, and death occurred. A few râles were present in the lungs before death.

A 6 year old brother, who was bitten by a tick at the same time, became ill two days after his sister was admitted to the hospital. He appeared to have the same disease, but recovered.

AUTOPSY FINDINGS

The skin showed innumerable petechiae, many of which had become confluent, forming large irregular splotches. The petechiae were most numerous in the locations in the order named, on the extremities, back, chest, abdomen, neck and face. There was also a generalized edema which was most marked in the neck and face, and particularly on the left side. Both eyes were chemotic and the lids swollen, the left being the more edematous. There was also more edema of the left arm and hand than of the right.

The peritoneal cavity contained nearly 300 cc. of clear yellow fluid. There were many small petechiae beneath the serosa of the intestines, and some large petechiae in the mucosa could be seen from the exterior. The abdominal lymph glands were enlarged and pink. The right pleural cavity contained approximately 40 cc. of clear

yellow fluid and the left pleural cavity 25 cc. There was a definite increase in pericardial fluid, about 35 cc. being present. The lungs showed rather numerous areas of bronchopneumonia. A caseous tubercle 3 mm. in diameter was found in the right lower lobe near the hilum. There were moderate numbers of petechiae in the mucosa of the stomach and small intestine; only a few were seen in the colon. No ulceration of the mucosa was seen. The liver showed little, other than a few small yellow tubercles. The heart, pancreas and adrenals appeared normal. The spleen was enlarged, weighing 100 gm., and the tissue was soft and deep purple in color. The kidneys were intensely congested, and several petechiae were seen in the pyramids, but none were seen in the cortex or pelves. The urinary bladder contained a few. The brain showed some flattening of the convolutions and intense congestion of the cortical vessels. The meninges and cerebrospinal fluid were normal. No abnormalities were seen on gross section of the brain.

MICROSCOPIC EXAMINATION

Heart: Some of the capillaries and many of the smaller arterioles are thrombosed. A few are occluded only by a hyalinized mass of fibrin, but included within the majority of the thrombi are polymorphonuclear and large mononuclear leukocytes. Many thrombi contain pyknotic nuclear fragments also. The surrounding tissue is infiltrated by large numbers of polymorphonuclear and large mononuclear leukocytes and plasma cells, and lesser numbers of lymphocytes (Fig. 9). The endothelial cells of many of the blood vessels are much enlarged, but only a few of the smaller vessels are occluded as a result of swelling of the endothelium. There are a great many leukocytes within the blood vessels of all tissues studied, and they are more numerous in the smaller vessels than in the larger ones. Many of the larger arteries show deposition of small masses of compact fibrin upon their inner walls, but no such vessel is seriously occluded by the thrombus — in fact these vessels show no reaction other than slight leukocytic infiltration about them. There is some myocardial edema. There are many foci in which a few myocardial cells are necrotic and invaded by polymorphonuclear and large mononuclear leukocytes. There are a great many foci not definitely associated with either acute myocardial necrosis or with thrombosis of the

blood vessels in which the interstitial tissue is infiltrated by large numbers of plasma cells and large mononuclear leukocytes and few lymphocytes. The endocardium and epicardium are normal.

Aorta: Normal.

Lungs: There are numerous areas of bronchopneumonia. Within three sections of lung are seen a few arterioles completely occluded by fibrin thrombi, and small, dense masses of fibrin are seen deposited upon the intima of a few other arteries.

Gastro-Intestinal Tract: Sections of the stomach, jejunum, ileum and colon show no mucosal lesions. However, within the submucosa and muscularis of each section there are several arteries which are partially or completely occluded by thrombi. The walls of these vessels are invaded by small numbers of leukocytes, some of which have pyknotic, fragmented nuclei. The endothelial cells of many of the unthrombosed vessels are swollen and the vessels contain many leukocytes. The tissue immediately about the thrombosed vessels and about some of the unthrombosed ones is infiltrated by plasma cells, large mononuclear leukocytes and a few mast cells; polymorphonuclear leukocytes are also seen in varying numbers about these vessels. An interesting finding observed to some extent in all tissues is the presence of pyknotic nuclear fragments within large mononuclear leukocytes in unthrombosed vessels. In the colonic submucosa are three longitudinally cut large arteries which have numerous thin patches of hyalinized fibrin deposited upon the intima; overlying the fibrin is a layer of thinned out endothelial cells, but there is no organization of the fibrin.

Abdominal Lymph Glands: Two small lymph glands are included in the section of stomach; the lymph sinuses are invaded by many large mononuclear leukocytes and polymorphonuclear leukocytes, many of which are phagocyted by the mononuclears. The germinal centers of some of the mesenteric lymph glands are hyperplastic. The sinusoids contain a few polymorphonuclear leukocytes, and many of these have been phagocyted by large mononuclears which are rather numerous. Within the reticulum of the glands are seen a few multinucleated cells only slightly larger than the average monocyte. Only a few thrombosed arterioles are seen within the glands, but within the surrounding connective tissue are many thrombosed arterioles with perivascular leukocytic infiltration.

Liver: There are a few tubercles in the sections, one of which is

calcified centrally. The liver cells have shrunk away from the sinusoidal walls and there is a small amount of granular precipitate in the resulting space. The sinusoids are much congested and contain many monocytes and polymorphonuclear leukocytes. The Kupffer cells are enlarged, and some show phagocytosis of erythrocytes and polymorphonuclear leukocytes. Some of the arterioles are partially or completely occluded by hyaline thrombi, and are surrounded by leukocytes. Small, hyaline, mural thrombi are seen in one portal vein and one hepatic vein. There is an infiltration of some of the portal areas by plasma cells and monocytes, even in the absence of arterial thrombosis. There are several foci of no constant distribution in which a few liver cells have become necrotic and invaded by polymorphonuclear leukocytes. A few of the liver cells contain fat vacuoles.

Pancreas: There are many foci in the pancreas in which the stroma is infiltrated by polymorphonuclear leukocytes, monocytes and plasma cells. Some of these are definitely associated with arterial thrombosis, but many are not. In many such areas there is also acute necrosis of the pancreatic alveolar cells, the insular tissue being uninjured. In addition to the numerous thrombosed small arteries, there are several large arteries which are partially, or completely occluded by fibrin. The fibrin in the partially occluded vessels is covered by a single layer of greatly attenuated endothelial cells and shows no organization.

Spleen: The splenic sinusoids are intensely congested and there are numerous areas of hemorrhage. There are many foci in which numerous polymorphonuclear and large mononuclear leukocytes are aggregated, and some of the sinusoids appear to be occluded by them. Some of the sinusoids are thrombosed. The monocytes show very active phagocytosis for red blood cells, polymorphonuclear leukocytes and lymphocytes. Although a few germinal centers show lymphoid hyperplasia the majority of the malpighian corpuscles are small, and there are few lymphocytes within the stroma.

Kidneys: There is moderate vascular congestion throughout the kidneys. Within the areolar connective tissue outside the pelvis and within the capsule there are numerous thrombosed arterioles with the perivascular infiltration previously mentioned. Within the pyramids are a great many foci of infiltration of the interstitial tissue by large mononuclear and polymorphonuclear leukocytes and

plasma cells. Most of these are definitely associated with thrombosed arterioles or capillaries. In the smaller and medium-sized foci there is no injury to the tubules (Fig. 11), and no leukocytes are seen within the tubules, but in the largest lesions some of the tubular epithelium is necrotic and the tubules are invaded by leukocytes. Some of the largest lesions have progressed nearly to the state of abscess formation (Fig. 10). Many of the larger lesions are associated with hemorrhage. Where the medullary rays are cut longitudinally it is seen that these foci of leukocytic infiltration radiate outward from the pelvis and are much longer in this diameter than they are transversely. The cortical tissue shows some parenchymatous degeneration, but is nearly free from lesions like those in the medulla. Except for a few focal lesions which are immediately adjacent to thrombosed vessels in the medulla or capsule, there are only two foci in the cortical tissue of two sections in which there is arteriolar thrombosis with perivascular leukocytic infiltration. One of these two thromboses is seen in the arteriole leading up to a glomerulus. One glomerulus is seen to contain a thrombus not associated with leukocytic reaction. Within some of the larger arteries of both cortex and medulla there are small masses of hyalinized fibrin covered by a thin layer of endothelial cells.

Urinary Bladder: The bladder shows the same changes that were found in the intestine, namely, thrombosis and perivascular leukocytic infiltration of the arterioles of the submucosa and muscularis.

Brain and Spinal Cord: A section of the lower thoracic spinal cord shows normal meninges, but within the fiber tracts are a few small, focal, perivascular cellular accumulations. There are two more such perivascular lesions at the extreme edge of one of the anterior horns; the cells are mainly neuroglia cells, but there are also some monocytes out in the tissue about the blood vessels (Fig. 4). Some of the vessels are thrombosed. A section of cerebral cortex shows a few polymorphonuclear and large mononuclear leukocytes within the pia-arachnoid (Fig. 12). Sections of cerebral cortex, optic thalami, pons, basal ganglia and olivary bodies show numerous examples of two apparently different types of lesions, and the sections of pons show a third type of lesion. The first type consists in the accumulation about blood vessels of large numbers of cells, predominantly neuroglial, although some monocytes are also present (Figs. 3 and 6). The endothelial cells of these vessels are swollen,

and some of the vessels are occluded, but many are not. The second type of lesion consists of occlusion of arterioles by polymorphonuclear and large mononuclear leukocytes, with infiltration of the surrounding tissue by some of these leukocytes (Fig. 5). The third type of lesion is a very striking one, and the lesions are much more extensive than the others. These are all in close association with partially or completely occluded arterioles, and consist in degeneration in an adjacent focus of the axones, which have become greatly swollen and nearly globular at points. Where the fiber tracts are cut transversely the appearance is that of large droplets (Fig. 2), but longitudinal section shows the "droplets" to be continuous with the fibers at each end (Fig. 1). Many of these globular swellings show a collection of very fine blue granules at one pole, and some show them at both poles. Occasionally one is seen which somewhat resembles a degenerated ganglion cell, but there cannot be the slightest doubt that practically all, if not all, of these structures are derived from axones. These structures show considerable variation in size, as reference to the plates will prove. Some large vacuoles are seen in the midst of these collections of degenerated axones, presumably resulting from complete degeneration and loss of the axonal material. That these lesions are areas of swelling of the axones is evidenced by the outward bowing of the normal fiber tracts which pass these areas and by the spaces at the edge of the lesions between the axones entering the lesions, as well as by the appearance of the lesions themselves. These lesions are less numerous than are the other two types, but are by no means rare. They appear to be miliary infarcts resulting from arterial thrombosis. In addition to the true perivascular cellular infiltration, many of the arteries within the brain tissue show an accumulation of monocytes in the stroma which accompanies them in their course (Fig. 13). Examples of this are most numerous in the pons, but are very abundant in the olivary bodies, basal ganglia and thalami.

Vertebral Bone Marrow: Not remarkable.

Skin: Sections of skin from the buttocks and thigh show small areas of hemorrhage in the corium, thrombosis of the capillaries and arterioles in the skin, and thrombosis of the large arteries in the subcutaneous fatty tissue. The walls of the vessels in the true corium show much damage and the media is infiltrated by monocytes and polymorphonuclear leukocytes. The tissue about the thrombosed

vessels contains many monocytes and polymorphonuclears, and a few plasma cells and mast cells. This perivascular leukocytic infiltration is most striking about the smaller arterioles and capillaries. Mast cells are seen in small numbers about the thrombosed vessels in other tissues, but are not as numerous as in the skin. Although the majority of the larger affected vessels in the subcutaneous fatty tissue are not completely thrombosed, many are, and many others which contain no thrombi are distended by blood. Some of the thrombosed vessels show hardly any leukocytic infiltration of their walls, but others have many leukocytes in their media and adventitia (Figs. 7 and 8). The thrombosed vessels are all arteries, the veins being apparently uninjured. Although no effort was made to secure serial sections, many sections from each block were examined and a satisfactory, although imperfect, approximation to a continuous series was thus obtained. In none of these sections is the perivascular leukocytic infiltration so widespread as to resemble the characteristic typhus nodules. The thrombi incorporate varying numbers of polymorphonuclear leukocytes and monocytes (endothelial leukocytes); in some thrombi the former predominate, and in others the latter are most abundant. Sections stained by Giemsa's method and by Goodpasture's carbol anilin fuchsin stain⁹ show *Rickettsiae* within endothelial cells and muscle cells of the media of thrombosed arteries (Figs. 14 to 19).

DISCUSSION

As justification for the diagnosis of Rocky Mountain spotted fever in this case several facts are offered. First, the history of tick bite followed by a disease which fits almost perfectly the picture of spotted fever described by Dyer and his coworkers, and which cannot be reconciled with their description of endemic typhus. Second, the rarity of typhus in children and the very low mortality rate of typhus also militate against the diagnosis of typhus. Third, the pathological identity of this case with those studied by Lillie. And fourth, experimental work by Dr. William Litterer, Director of the Division of Laboratories of the Tennessee State Health Department.¹⁰ This work has not been completed, but offers conclusive immunological evidence that the child did not have typhus. Blood taken during the last days of the patient's illness was injected intra-

peritoneally into guinea pigs, and the strain of virus has been maintained to date by passage through guinea pigs. All of the animals given this disease have died; however, up till now only a relatively small number of animals has been used. Guinea pigs which have recovered from an attack of typhus induced by inoculation with a strain of endemic typhus virus maintained in the laboratory are not immune to the spotted fever virus from this case. The reciprocal cross immunity has not yet been done.

The lesions found in this case are almost identical with those described by Lillie, differing in only a few minor details. Thrombosis and perivascular leukocytic infiltration in the mucosa and submucosa of the gastro-intestinal tract was present in this case and absent in the one case of Lillie's in which the gastro-intestinal tract was examined. In our case a few arterioles in the lungs contained thrombi. This was not seen in Lillie's cases. The only important difference is in the renal lesions. In Lillie's cases there were many foci of perivascular lymphocytic infiltration; in our case the predominant cells in the perivascular accumulations were polymorphonuclear and large mononuclear leukocytes.

The brain lesions observed in this case are identical with those described and illustrated by Lillie, and with the exception of our third type (Figs. 1 and 2) are also identical with those noted by Wolbach¹¹ in his study of European typhus. This third type of lesion has not been seen in an incontrovertible case of typhus. It was apparently seen by Pinkerton and Maxcy¹² in a case diagnosed as endemic typhus, but it seems probable that their case was actually one of Rocky Mountain spotted fever instead.

This third type of brain lesion appears to be characteristic of the Eastern form of Rocky Mountain spotted fever, and may prove to be a reliable histological criterion for the differential diagnosis of the two diseases. The occurrence of brain lesions in the Eastern form of the disease is apparently constant and constitutes an important difference between the Eastern and Western forms. These points can be clarified only by the study of more brains from human cases of the two forms of this disease.

Demonstration of *Rickettsiae* in sections of skin was first accomplished with Giemsa's stain, using Wolbach's technique.⁶ Since Goodpasture's carbol anilin fuchsin stain had been found to give such excellent results with other microorganisms, we wished to see

how well it would work with *Rickettsiae*. Wolbach tried this stain with the technique originally used and found that it gave fair results, but was confusing because of intense staining of other material occurring in the lesions. Goodpasture subsequently improved the technique,⁹ and this modification was employed in the present study. If this technique is strictly followed it will be found that it will be necessary to run the sections through more than half a dozen times in order to stain the organisms deeply; for this reason the sections were stained for four minutes or more before decolorization, and running them through four times gave very intense staining of the organisms. If tissues other than skin are stained by this method they must not be stained for much more than a minute, for otherwise they cannot be sufficiently decolorized. This method gave very satisfactory results with the skin sections, the organisms within the muscle cells standing out strikingly, and all the photomicrographs of the organisms were made from sections stained by this method. For demonstration of the organisms within the muscle cells, and according to Wolbach they are found most abundantly in the smooth muscle cells of the media of vessels with the lesions, this method gives results fully equal to, and in our opinion superior, to the results obtained with Giemsa's stain. Furthermore, the sections may be stained in much less time (less than an hour). For demonstration of the organisms within endothelial cells it is not so satisfactory, for the reason given by Wolbach. However, search of several sections will hardly fail to show a few endothelial cells in thrombosed vessels in which there is no question as to the presence of the organisms. One familiar with the appearance of *Rickettsiae* and familiar with the staining method will find it an excellent stain, but it cannot be recommended as one to be used routinely to replace Giemsa's stain in the study of Rickettsial diseases, although it is a stain which may be used profitably to supplement Giemsa's method.

In addition to the two cases of Rocky Mountain spotted fever already mentioned in this paper, three other cases occurred in Tennessee in 1931. All five cases occurred within a relatively short distance of Nashville, and all the cases developed shortly after tick bite. In two cases the diagnosis was purely clinical, but the other three cases were shown by immunological tests not to be typhus. There was only one death in this series of cases.

SUMMARY

1. The occurrence of Rocky Mountain spotted fever in Tennessee and a histological study of a fatal case are herewith reported.
2. The occurrence of brain lesions in the Eastern form of Rocky Mountain spotted fever reported by Lillie is confirmed.
3. Attention is called to a type of brain lesion which may prove to be diagnostic of the Eastern form of Rocky Mountain spotted fever.

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DESCRIPTION OF PLATES

PLATE 15

- FIG. 1. Pons. Longitudinal section of focus of axonal degeneration. A thrombosed arteriole containing many polymorphonuclear and large mononuclear leukocytes is seen at the inferior edge of the lesion. Phosphotungstic acid hematoxylin stain. $\times 200$.
- FIG. 2. Pons. Cross-section of a similar area. A thrombosed arteriole is seen 2 cm. to the left of the middle of the photograph. Phosphotungstic acid hematoxylin stain. $\times 325$.

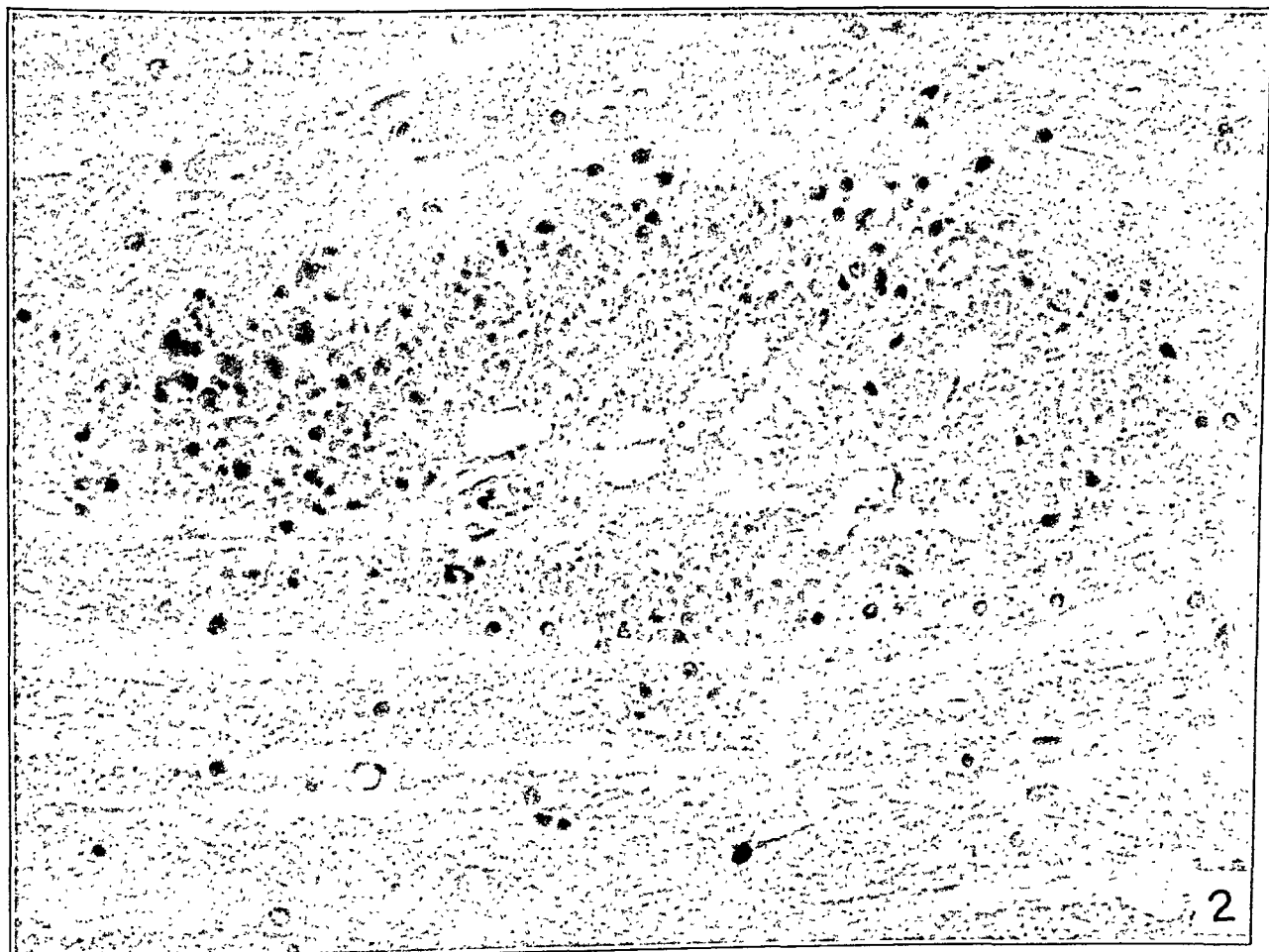
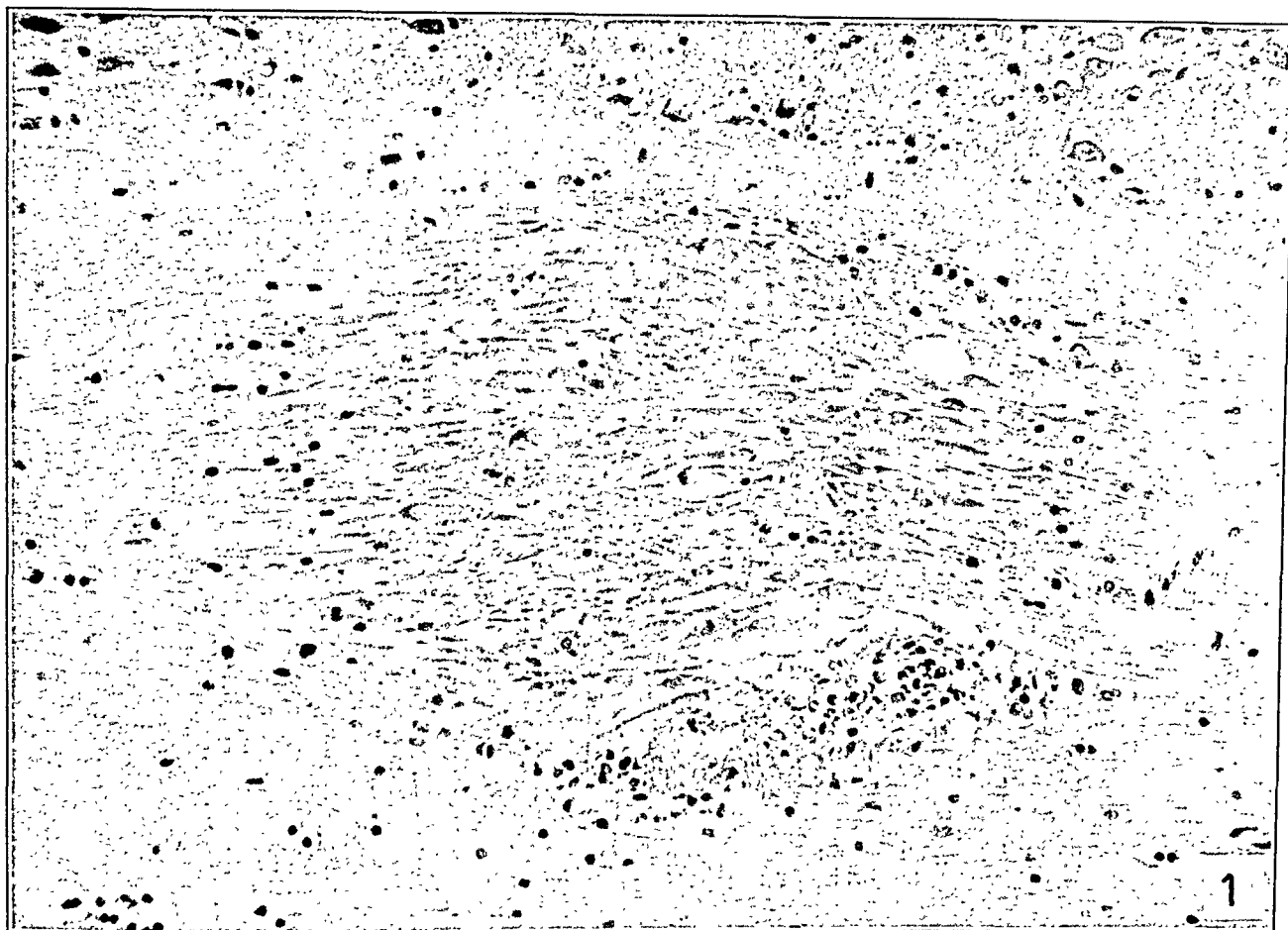


PLATE 16

- FIG. 3. Pons. This field shows thrombosis of an arteriole at the left, perivascular cellular infiltration, and leukocytic infiltration of the pia about the blood vessel. Phosphotungstic acid hematoxylin stain. $\times 200$.
- FIG. 4. Spinal Cord. Perivascular lesion with glia cells and some large mononuclear phagocytes. Hematoxylin and eosin stain. $\times 350$.
- FIG. 5. Pons. Thrombosed arteriole with polymorphonuclear and large mononuclear leukocytes. Note cellular debris within the leukocytes. Phosphotungstic acid hematoxylin stain. $\times 550$.
- FIG. 6. Pons. Perivascular lesion of typhus type. Phosphotungstic acid hematoxylin stain. $\times 550$.

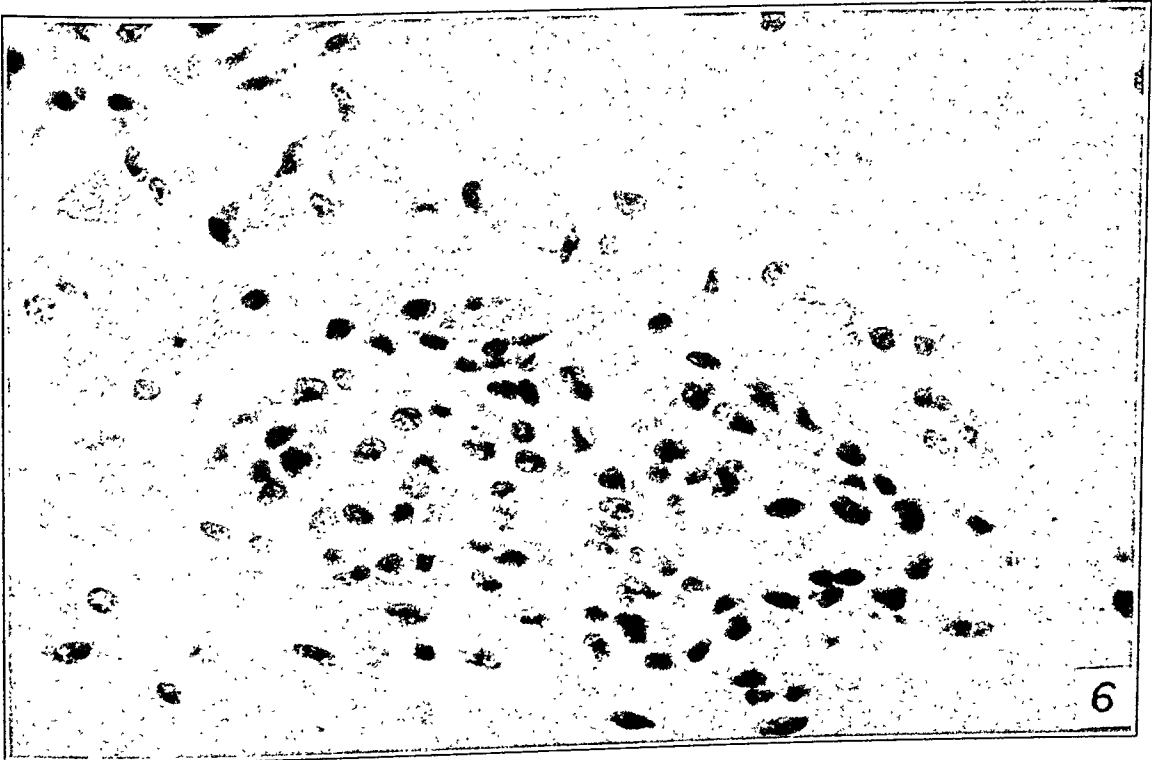
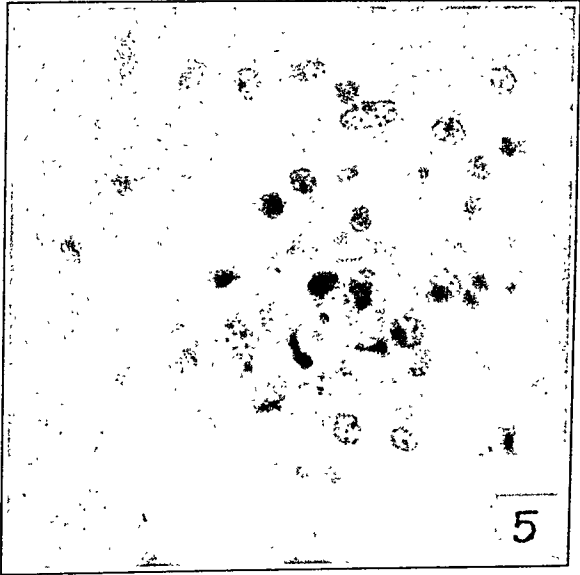
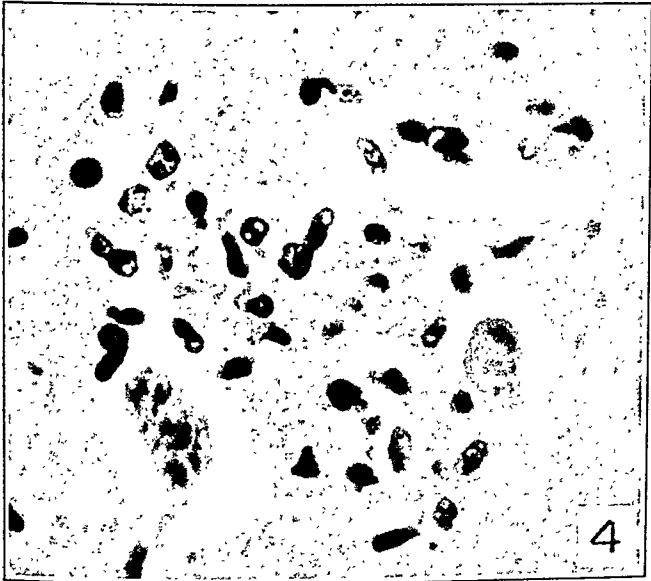
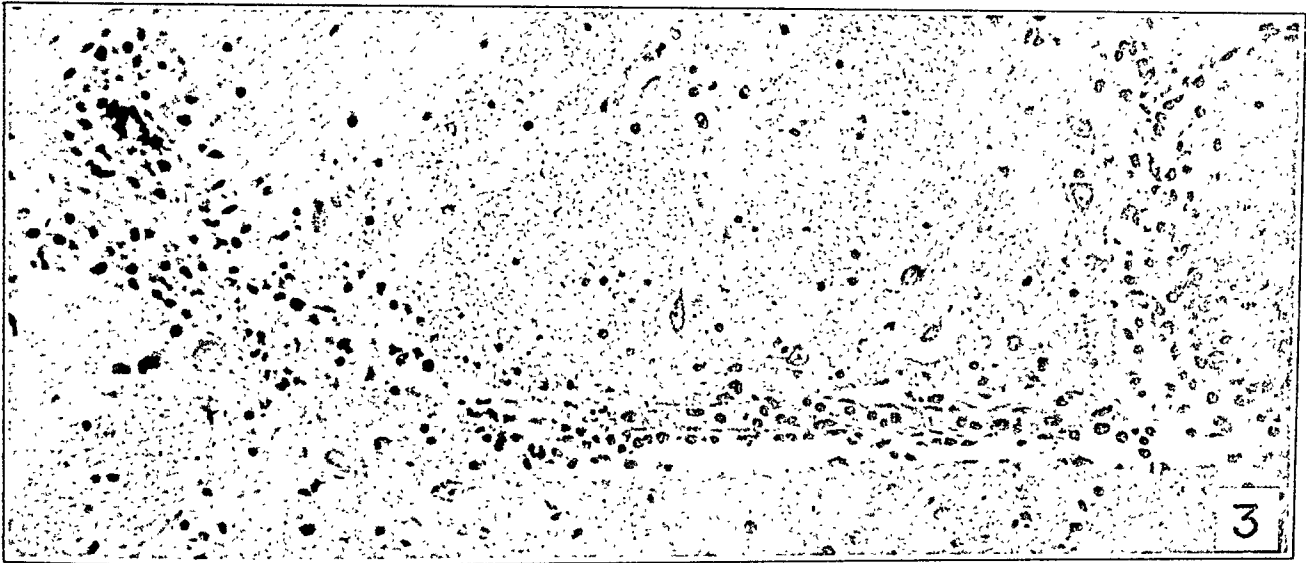


PLATE 17

FIG. 7. Artery in subcutaneous fatty tissue. Note thrombosis, extensive leukocytic invasion of the media, and very slight perivascular infiltration. $\times 100$.

FIG. 8. Artery in subcutaneous fatty tissue. Note thrombosis and leukocytic invasion of the vessel wall. There is far more leukocytic invasion of the tissues about the vessel than is usually seen. Hematoxylin and eosin stain. $\times 325$.

FIG. 9. Heart. In addition to the marked interstitial leukocytic infiltration several thrombosed arterioles and capillaries are seen. Hematoxylin and eosin stain. $\times 375$.

FIG. 10. Kidney. One of the larger lesions is shown here. There is extensive leukocytic invasion with necrosis of tubules. Two thrombosed vessels are seen, one near the middle of the photograph, and the other half way between this and the lower right corner. Hematoxylin and eosin stain. $\times 225$.

FIG. 11. Kidney. One of the smaller lesions. Note the thrombosed vessel with extensive leukocytic infiltration of the interstitial tissue and absence of damage to the tubules. Hematoxylin and eosin stain. $\times 325$.

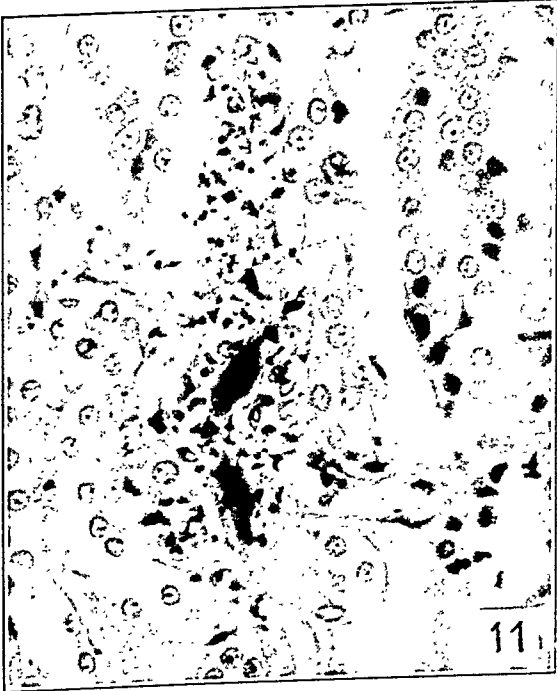
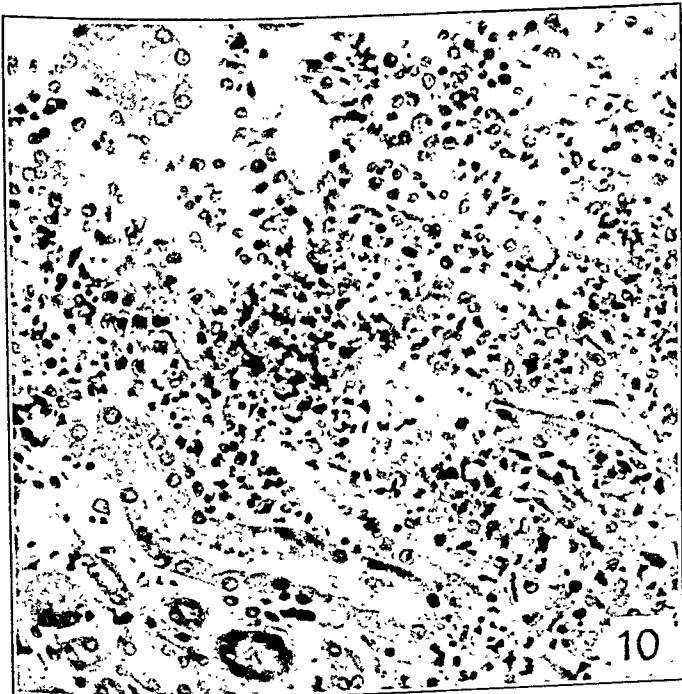
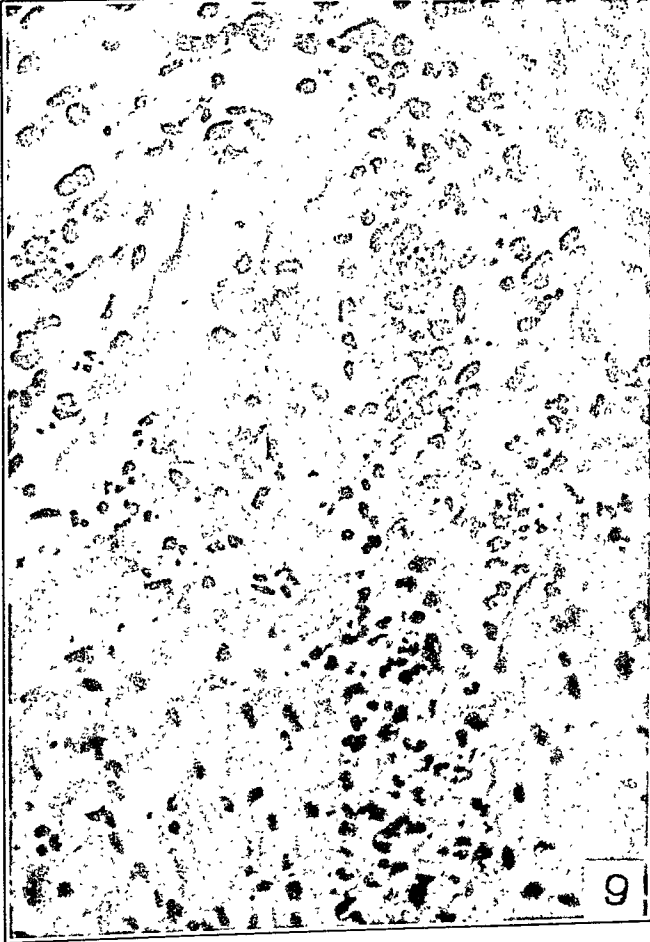
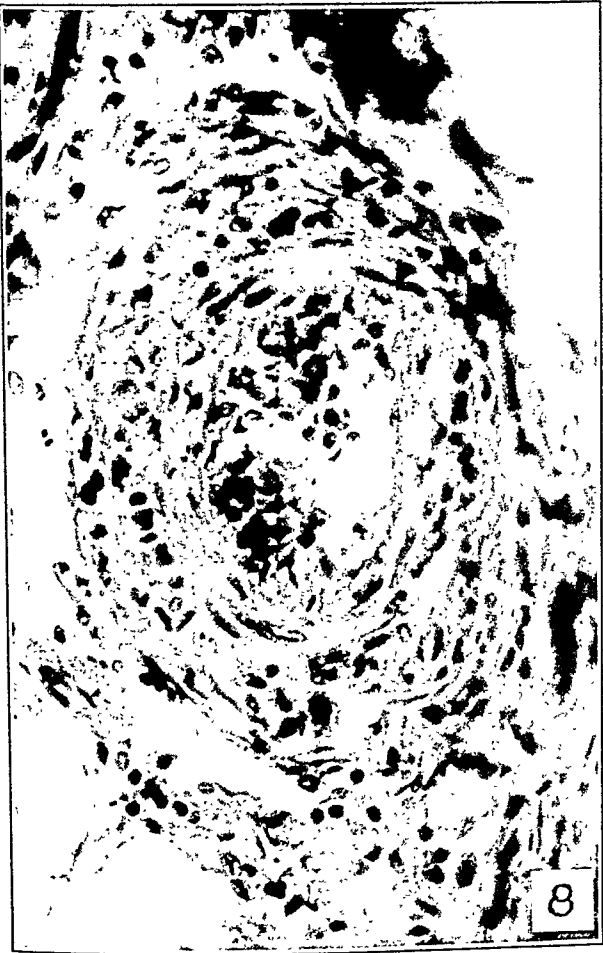


PLATE 18

FIG. 12. Cerebral meninges. This photograph shows clearly the types of cells appearing in the exudate. Hematoxylin and eosin stain. $\times 250$.

FIG. 13. Artery in region of olivary bodies. This shows the type of cell infiltrating the pial vestment of the arteries. Hematoxylin and eosin stain. $\times 375$.

FIG. 14. Group of *Rickettsiae* within muscle cell of media. A definite halo may be seen about most of the organisms. A portion of thrombus is seen at the lower left.

FIG. 15. Two groups of *Rickettsiae* within two different muscle cells.

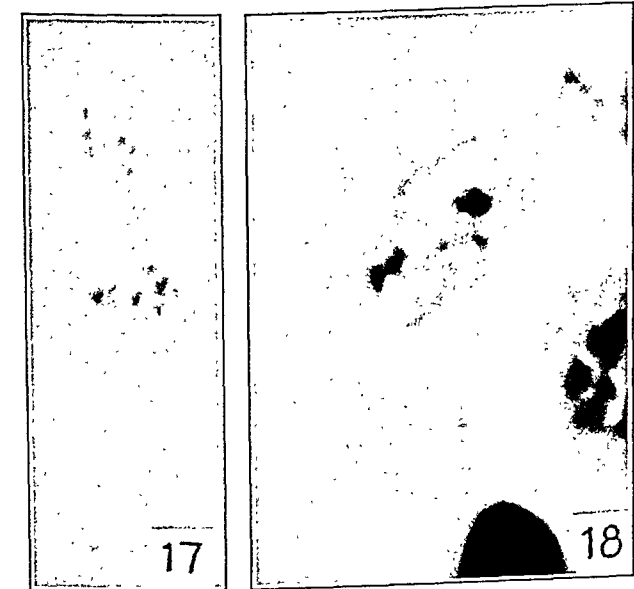
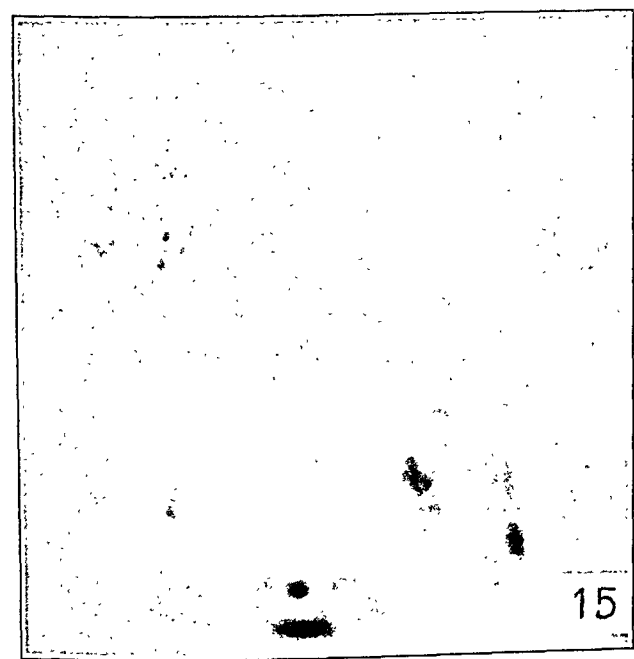
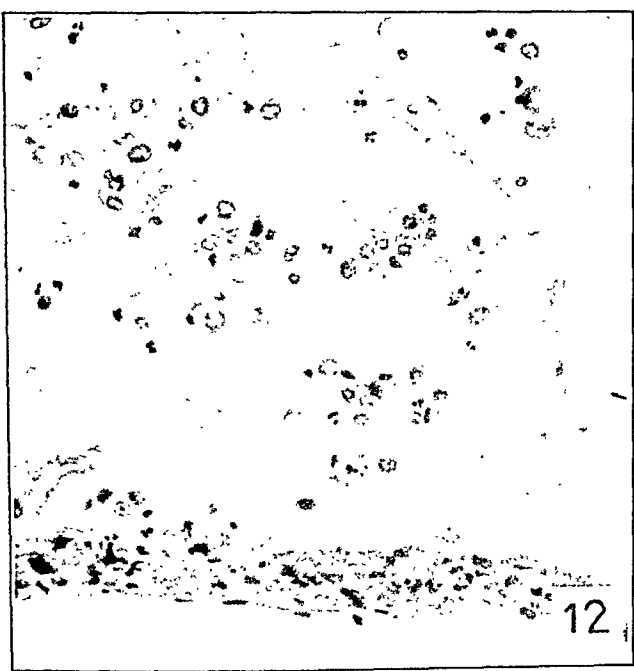
FIG. 16. Group of *Rickettsiae* within a muscle cell. A portion of thrombus is seen below.

FIG. 17. The same field seen immediately above in Fig. 15, but focused at a slightly different level to show groups of *Rickettsiae* within adjacent muscle cells.

FIG. 18. Endothelial cell containing two groups of *Rickettsiae*, one above the nucleus, and one below. Those above the nucleus are surrounded by a very distinct halo.

FIG. 19. Two groups of *Rickettsiae*. One group within an endothelial cell below the nucleus, and the other group within a muscle cell to the left of the endothelial cell nucleus. Several of these organisms are surrounded by a faint halo.

(FIGS. 14 to 19 are photomicrographs of *Rickettsiae* found in the walls of thrombosed arteries in the skin. All were stained by Goodpasture's carbol anilin fuchsin stain and are magnified 2700 diameters, except Fig. 19 which is magnified only 2000 diameters.)





METASTATIC CALCIFICATION OCCURRING IN MYELOGENOUS LEUKEMIA *

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Metastatic calcification is a term originally employed by Virchow to designate a condition in which there is mobilization of the body calcium from its primary depots in the bones and redistribution to other tissues and organs of the body. Virchow's original inferences that there was oversaturation of the blood with calcium derived from the bones and redeposition in distant sites of the body found universal acceptance because the condition usually accompanied disease in which there was extensive bone destruction, such as osteomyelitis, tuberculosis of bone, primary and metastatic tumors, and leukemias.

Wells¹ reviewed the subject of metastatic calcification, and earlier² compared the processes of calcification and ossification. He reported a case of myelogenous leukemia in which calcium metastasis occurred and quoted the experiments of Tanaka³ and others, who reproduced the condition in animals after intraperitoneal injections of calcium salts. In a careful review of the literature he discovered twenty-nine cases of this condition, to which he appended one of his own. The following, it is believed, is the second to be recorded in the American literature.

REPORT OF CASE

Clinical History: The patient, F. L., a girl 19 years of age, unmarried, native born, was admitted to the Fourth Medical Division of Bellevue Hospital on Dec. 5, 1931. Her chief complaints were weakness and general malaise, loss of appetite and weight, and enlargement of the abdomen of a year's duration. The family history was essentially negative. As a child the patient had always been frail and of delicate health, but had had only the more common childhood diseases such as measles and diphtheria. On Feb. 24, 1931, she had been admitted to another hospital complaining of an itching rash of two weeks' duration.

* Received for publication July 25, 1932.

At that time the spleen was enlarged and the blood picture was characteristic of myelogenous leukemia.

Physical Examination: Patient appeared as a frail white girl, extremely anemic, underweight and chronically ill. The skin and mucous membranes were pale, the teeth in good repair, the tonsils small. The neck showed a small discrete adenopathy of the angular and supraclavicular lymph nodes. The heart and lungs were essentially normal. A large mass, undoubtedly spleen, occupied the entire left half of the abdomen from the xyphoid process to the crest of the ileum. This was not tender. The liver was enlarged to two fingers' breadth below the costal margin. Extremities showed muscular wasting but no edema. Small, discrete, bilateral inguinal lymphadenopathy was present.

Laboratory Findings: The blood pressure remained about 100/60 throughout the course of illness. The urine always showed considerable albumin and many white blood cells. Wassermann test negative. Blood total nitrogen 30, sugar 79 mg. per 100 cc. Basal metabolism on December 15 was + 75. On admission total white blood count was 21,200, 63 per cent immature forms, 22 per cent adult polynuclear neutrophiles, 2 per cent eosinophiles, 13 per cent lymphocytes, and the red blood count 2,200,000.

Course of Illness: On December 7 sodium cacodylate, 1 gm. daily was started.

On December 8 the blood showed 38 per cent hemoglobin, 3,080,000 red blood cells, 37,000 white blood cells. Smear showed many normoblasts and a predominance of young granulocytic forms.

December 16 the patient was very weak and complained bitterly of abdominal and lumbar pain which required large and frequent dosages of codein. The blood picture remained essentially unchanged.

December 19 sodium cacodylate was discontinued because the patient had developed a garlic odor to the breath. Dullness and moist râles were present at the base of left lung.

December 22 the patient received a transfusion of 500 cc. of whole blood.

On December 24 the total white blood count rose to 66,000.

December 29 radiation therapy was started. This was repeated once on December 31. At this time there was pronounced mental lethargy.

On Jan. 1, 1932, the patient was extremely toxic and comatose. She presented a picture of terminal failure characterized by embryocardia, diffuse chest râles and coma. A blood count just before death showed 1,200,000 white blood cells, of which 92 per cent were granulocytes.

Death occurred at 6.00 A.M. Jan. 2, 1932.

AUTOPSY REPORT

Autopsy was performed three hours after the patient's death. The anatomical diagnoses were: myelogenous leukemia with involvement of the spleen, liver, kidneys, retroperitoneal and mediastinal lymph nodes, and the superficial supraclavicular and axillary lymph nodes; metastatic calcification of the left auricle, pulmonary veins, aorta and main pulmonary artery, and of the renal, coeliac, mesenteric and orifices of the coronary arteries.

The body was that of a young, adult, white female, who appeared about 15 years of age, of delicate slender frame, moderately emaciated. Body length 158 cm., weight 95 lbs. Skin smooth, pale and white. Nodular masses about $1\frac{1}{4}$ cm. in diameter were palpable in both supraclavicular fossae and in the axillae.

Subcutaneous fat was absent over the thorax and slight over the abdomen. Recti muscles were delicate and poorly preserved. The spleen occupied the entire left side of the abdominal cavity from the dome of the diaphragm above to the pubic symphysis, displacing the small intestines to the right side. The superior anterior surface of the spleen was adherent to the posterior surface of the left lobe of the liver. The transverse colon dipped posteriorly to the upper half of the spleen, which appeared to compress it at that point.

The thoracic cavity was essentially normal.

Heart: Weight 250 gm. Right auricle and ventricle contained large masses of chicken-fat clot. Left auricle contained similar clot. Its endocardial surface was raised, thickened and had a chalky white color. The valves were normal. The endocardium in the sinuses of Valsalva of both pulmonary and aortic valves was thrown into a series of parallel longitudinal ridges about 1 mm. in length. These ridges occurred in the mouths of the coronary arteries. The entire length of the aorta showed at regular intervals a succession of these parallel ridges. The various visceral arteries, splenic, coeliac, mesenteric and renal, were thrown up into these same ridges which felt brittle and often involved the entire cross-section of the vessel.

Lungs: Right lung weighed 750 gm., the left lung 650 gm. The visceral pleura was pale pink and glistening. The lungs were generally crepitant, save for areas in the periphery where small scattered infarcts were present. When sectioned, a grating sensation was imparted to the knife. The vessels were gray, felt calcified and projected a few millimeters from the cut surface. The apertures were frequently thrombosed and often gaping.

Liver: Greatly enlarged, weighing 2750 gm., and measuring 35 by 22.5 by 15 cm. The color was dark reddish brown. Capsule smooth, edges sharply defined. On section consistence slightly increased. Small, linear, pale areas were visible throughout the cut surface, especially beneath the capsule.

Spleen: Greatly enlarged, weighing 2550 gm., measuring 37.5 by 15 by 10 cm. Splenic artery showed calcific changes as described.

There were two shallow notches at the mesial border and a large, solitary, wedge-shaped infarct just beneath the hilum. On section consistence firm, surface red and coarsely granular.

Kidneys: Combined weight 400 gm. The capsule stripped readily, surface smooth, shiny and the color of chicken-fat. On section cortex slightly swollen and the cortical markings practically obliterated.

Lymphatics: The perigastric, posterior mediastinal, superior and inferior periaortic and iliac nodes, as well as the superficial groups, were enlarged. The retroperitoneal nodes varied from 1 to 6 cm. in diameter, and were usually discrete, save for the periaortic groups which were fused. Cut surface had the appearance of fish meat.

MICROSCOPIC EXAMINATION

Tissues from the various organs were removed and fixed for 48 hours in 10 per cent formol solution, after which sections were embedded in paraffin and stained with hematoxylin and eosin, by Giemsa's method and with Weigert's elastic tissue stain. Smears of the bone marrow were stained with Wright's blood stain.

The bone marrow smears show fairly typical myeloid transformation with every variety of granulocyte present, particularly the early forms.

The liver, spleen and lymph nodes show characteristic leukemic deposits.

The endocardium of the left auricle is the site of extensive calcium deposition which is sharply limited to the subendothelial elastic fibers. These are swollen, often ruptured and impregnated with calcium salts, assuming an intense purple stain with hematoxylin. The elastic fibers are separated by numerous necrotic cells, probably leukocytes, themselves staining deeply for calcium. The deeper layers contain numerous neutrophilic leukocytes spreading apart the auricular myofibrils. The vasa vasorum of the myocardium and epicardium contain leukemic thrombi.

The arterial changes are best demonstrated in the aorta. Hematoxylin and eosin-stained sections demonstrate that a selective calcification is confined almost exclusively to the inner elastic lamina, which like the elastic fibers of the auricle described above, is swollen, stains deeply with hematoxylin and is thrown into exaggerated folds corresponding to the macroscopic ridges. With Weigert's elastic

tissue stain some of the elastic fibrils are seen to be ruptured. Sections of the main pulmonary artery and of the smaller visceral arteries, particularly the splenic, reveal identical changes. In every case the grossly visible ridges in the vessel walls are seen to correspond to points of rupture of the calcified elastic laminae.

Lung sections reveal a uniform, widespread and pronounced dilatation of the pulmonary veins by leukemic thrombi. In sections these distended vessels are so prominent as to comprise almost one-fifth of the area of a cross-section. The intima of these veins shows widespread calcification and fragmentation. Occasionally the calcified endothelium and subendothelial tissue is desquamated or partially detached from the vein wall. Where points of rupture of the elastica are present, the veins are thrown into bizarre patterns. A few arterioles show similar changes but the venous changes are predominant.

The interalveolar capillaries are distended with leukemic blood. Oftentimes the interalveolar septa are calcified and ruptured. No calcium is found deposited in the bronchial epithelium or cartilage.

The kidneys show leukemic infiltration of both cortex and medulla. Many of the tubules and ducts are converted into deep purple-staining masses of calcium. Often rings of calcium occupy the lumen. Elsewhere the lining cells show calcification of the intracellular substances and detachment from the basement membrane. Occasionally, calcium is found in the glomerular tufts and in the space between capsule and tuft epithelium. The medullary ducts contain numerous leukocytes, many of which stain for calcium.

DISCUSSION

The lesions described above are fairly typical of those to which the term "metastatic calcification" has been applied. Metastatic calcification differs from a number of types of pathological calcification in that previous degenerations or necroses of tissue are not essential prerequisites. It is best regarded as a fundamental disturbance of calcium equilibrium in which calcium is mobilized from the depots in the long bones and reprecipitated in other organs and tissues of the body.

As Wells pointed out, the elastic tissues of the body, especially the elastic laminae of systemic arteries, are favorite sites for calcium

metastases. Wells believes that this predeposition of the elastic tissues resides in the physical constitution of elastic fibrils, which under certain conditions are capable of adsorbing soluble calcium salts and reprecipitating them. Change of alkalinity of the tissue fluids probably favors precipitation. He pointed out, too, that those organs which most frequently show this phenomenon generally excrete acids, whereupon there is a decrease in the acidity of the remaining tissue fluids — a reaction favoring precipitation of calcium salts. Thus, the lungs excrete carbonic acid, the gastric mucosa hydrochloric acid, the kidneys acid phosphates. Hofmeister⁴ showed that the occurrence of metastatic calcification in the gastric mucosa was limited to interglandular spaces in the region of the fundus where the acid-secreting glands are located.

In the lungs where CO_2 is excreted, the calcification, as would be expected, occurs in the alveolar septa and in the veins. In the left auricle and in the peripheral blood vessels where the CO_2 tension of the blood is diminished, metastatic calcification is also found.

Where metastatic calcification has been encountered it has invariably occurred in bone diseases, primary and secondary neoplasms, suppurative processes and in leukemias. All of these conditions are characterized in common by osteolytic bone changes. Osteolysis, while it is uncommon, does occur in leukemias. Craver and Copeland⁵ have recently made roentgenographic studies of the bone changes in leukemias. Of 100 cases of lymphatic leukemia and 83 cases of myelogenous leukemia analyzed by them, 3 of the former and 1 of the latter showed definite roentgenographic evidence of bone involvement. The X-ray picture usually showed cortical destruction with punched-out areas of osteoporosis. The bones found most frequently involved were usually the pelvis, humerus, ulna, radius, spine and skull. The infrequency of these osseous lesions in the leukemias would correspond to the rarity of metastatic calcification as a postmortem finding.

Conditions indistinguishable from metastatic calcification have recently been reproduced in experimental animals by injection of parathormone (Collip)^{6,7} and by the prolonged ingestion⁸ of radiated ergosterol. Both these substances elevate the concentration of blood calcium. The source of the increased blood calcium following parathormone injections is undoubtedly the long bones, as has been shown by Bauer, Aub, and Albright,^{9,10} who found that loss

of trabeculae in the long bones of rabbits resulted after successive increasing dosages. That parathormone is capable of elevating blood calcium, even on a calcium-free diet, has been demonstrated by Hess and Lewis ¹¹ and others. A marked hypercalcemia results when these animals are fed a normal diet, however. The mechanism whereby ergosterol and its allies produce hypercalcemia and metastatic calcification is unknown, though the fact that these substances may stimulate the parathyroid glands has been advanced as a likely explanation of their action.

The writer has observed calcification of the pulmonary alveolar septa indistinguishable from that in the above case in a patient with chronic diffuse glomerulonephritis who died in uremia. Wells quotes Schmidt ¹² who described calcification of the myocardium of the left ventricle, pericardium, lungs, kidneys, pulmonary veins, stomach and systemic arteries in a patient with advanced nephritis. It appears not unlikely that such forms of calcification are the same as those of the metastatic variety.

SUMMARY

1. A case is reported of metastatic calcification occurring in a young girl whose history indicated that she had had myelogenous leukemia for at least two years.

2. The term is employed to designate a condition in which calcium is mobilized from the depots in the bones and reprecipitated in other tissues of the body, particularly elastic tissues, which are probably physically constituted to favor calcium adsorption and reprecipitation. Calcium deposition is also seen to be favored by any phenomenon which reduces the acidity of the tissue fluid. Hence, metastatic calcification occurs in those tissues where a change in the alkaline direction very likely occurs.

3. Recent evidences mentioned show that the leukemias are rarely associated with osteolytic changes in the skeleton and this infrequency probably explains the rarity of metastatic calcification as a finding in leukemias.

4. Finally, the identity of this condition with the calcifications occurring after administration of parathormone and irradiated ergosterol is reaffirmed.

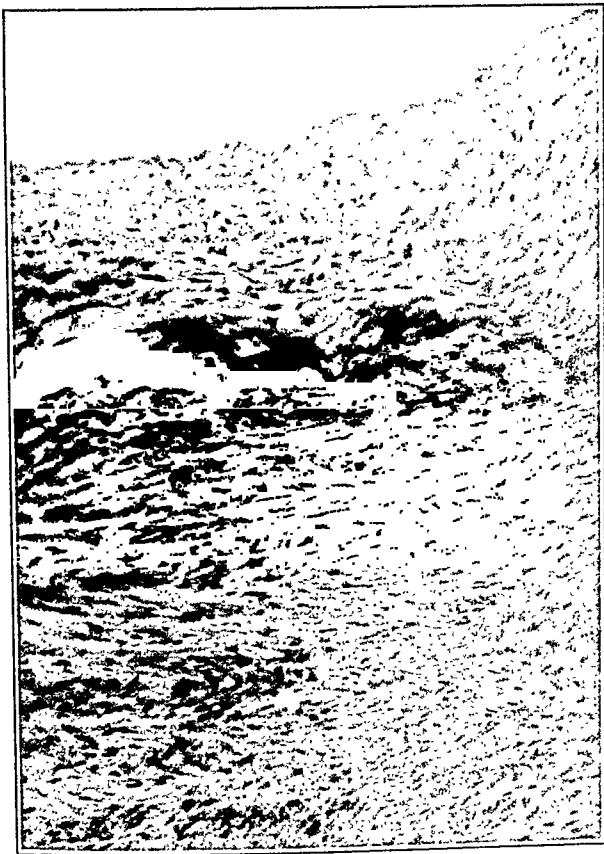
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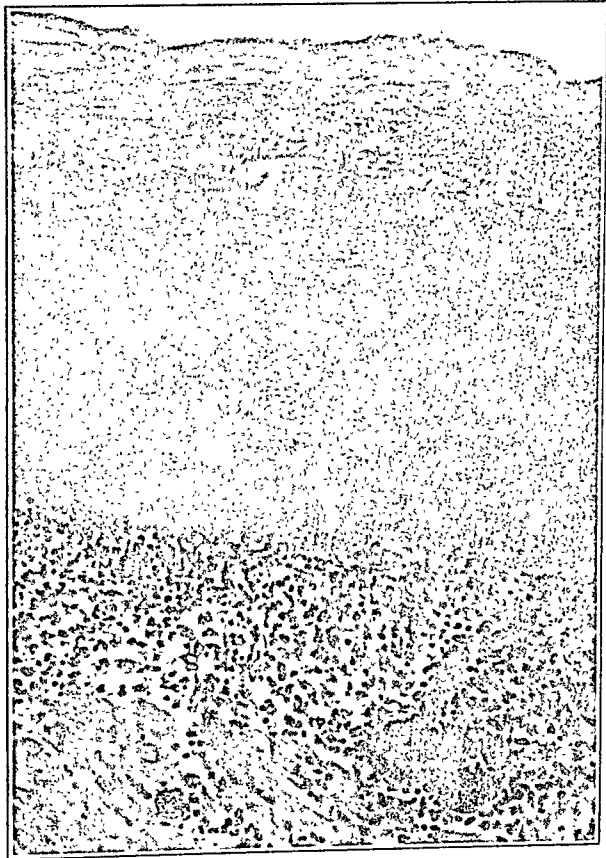
DESCRIPTION OF PLATE

PLATE 19

- FIG. 1. Photomicrograph of aorta showing selective calcification of the inner elastic lamina. Hematoxylin-eosin stain. $\times 100$.
- FIG. 2. Photomicrograph of left auricle. Note the leukemic infiltration. The dense black areas represent areas of calcified elastic tissue fibrils. Hematoxylin-eosin stain. $\times 120$.
- FIG. 3. Photomicrograph of lung. Note the dense calcification of the pulmonary veins and of the interalveolar septa. Numerous points of rupture of the calcified elastica are seen. Weigert's elastic tissue stain. $\times 100$.
- FIG. 4. Photomicrograph of kidney showing leukemic infiltration and calcification of some of the tubules. Hematoxylin-eosin stain. $\times 120$.



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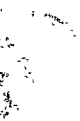
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MYELOBLASTIC SARCOMA OF THE SCAPULA ASSOCIATED WITH CHRONIC SPLENOMYELOGENOUS LEUKEMIA *

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Hyperplasia of the hemopoietic tissues of bone marrow in myelogenous leukemia has prompted many speculative considerations of the disease. Some propose that leukemias are tumors, but the arguments favoring or contradicting this interpretation of the disease are indecisive. A patient after many years illness with chronic myelogenous leukemia occasionally has a growth of hemopoietic tissues in a bone and, because of local destructive extension and metastases into the viscera, the neoplasm has the characteristics of a malignant, marrow tissue tumor. Such conditions are described in this report. The myelogenous leukemia had been recognized for four years. The destructive tumor of the left scapula, and metastases in the axillary, biliary and cervical lymph nodes, the liver, pleura, spleen and twelfth thoracic vertebra, were demonstrated by the postmortem examination.

REPORT OF CASE

Clinical History: A white woman, aged 58 years, was in the care of Dr. Ralph C. Larrabee, Boston, from October 1927 until January 1930 for chronic splenomyelogenous leukemia. When first examined by Dr. Larrabee the patient's blood contained 2,792,000 erythrocytes, 312,800 leukocytes, 496,000 platelets per cmm. and the hemoglobin was 52 per cent. The distribution of the leukocytes was as follows: 55 per cent neutrophilic polymorphonuclear leukocytes, 5 per cent lymphocytes, 2 per cent mast cells, 37 per cent neutrophilic and 1 per cent eosinophilic myelocytes. The patient reacted well to roentgen therapy over the spleen and on Dec. 19, 1927, the hemoglobin was 74 per cent and the leukocytes 18,600 per cmm. of blood. Evidence of leukemia was almost absent except for the persistence of many mast cells. During the spring of 1928, the leukemic condition of the blood returned and the patient became refractory to roentgen therapy. By October 1928, the hemoglobin was 44 per cent, the red cells 2,312,000 and the white cells 409,600 per cmm. Of the leukocytes 40 per cent were neutrophilic and 1 per cent eosinophilic polymorphonuclear leukocytes, 4 per cent were mast cells, and 48 per cent neutrophilic, 2 per cent cosino-

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philic and 3 per cent mast cell myelocytes. Although three roentgen treatments over the spleen were ineffective, three over the long bones (tibiae) caused marked improvement. By October 1929, she had again relapsed and roentgen therapy was not effective. Her general condition was unfavorable.

In January 1930, she came under the care of Dr. Joseph A. Capps, Chicago, Ill. The blood had about 1,000,000 each of white and red blood cells per cmm. and the hemoglobin was 20 per cent. The spleen was especially large. At St. Luke's Hospital roentgen radiations of the chest dorsally were continued twice monthly during the winter and summer of 1930, and the patient's condition improved. The spleen decreased to about one-half its former size and on March 31, 1930, the blood elements were 3,200,000 erythrocytes, 49,000 leukocytes, and 61 per cent hemoglobin. Later the leukocyte count varied between 78,000 and 117,600, which it attained in September 1930. The following month the patient's physician in Boston found her spleen not enlarged, and the blood with 4,040,000 erythrocytes, 8400 leukocytes per cmm., and the hemoglobin 75 per cent. In the differential count of 100 leukocytes, 1 myelocyte, 10 eosinophils and 11 mast-cells were the only abnormal cells found. The patient had gained considerable weight and felt well.

During the night of Jan. 26, 1931, she awoke with a severe pain in her back. The following day the lumbar spine was stiff to motion in all directions. Although there was no tenderness, an infarct of the spleen was considered probable. Subsequently there were several attacks of pain in the left shoulder, notably one on March 18, 1931, severe enough to require opiates for relief. On June 8 of that year, because of continued distress, X-ray pictures were taken of the left shoulder by Dr. George W. Holmes at the Massachusetts General Hospital. He noted moderate atrophy of the bones about the joint, but no evidence of tumor. On July 19, 1931, at the age of 62 years, she entered St. Luke's Hospital, Chicago, because of extreme general weakness and occasional recurrences of the pain in the left arm and shoulder. Emaciation, a huge spleen and a rough systolic murmur over the cardiac apex were the essentials of the physical examination.

The blood contained 2,290,000 erythrocytes, 18,900 leukocytes per cmm., and the hemoglobin was 39 per cent. Among 100 leukocytes there were 2 lymphocytes, 12 neutrophils, 3 eosinophils, 20 band-shaped polymorphonuclear leukocytes, 46 myelocytes, 13 metamyelocytes and 4 myeloblasts. The white cells continued to increase gradually to 42,000 per cmm., but dropped to 26,350 on August 17. The chief complaint was continuous pain in the left arm and occasionally in the left shoulder. On August 9 these tissues became swollen, indurated and tender, and a few small pale nodules appeared along the inner bicipital groove of the left arm. The temperature had ranged from 99 to 102,^o but on August 19 it rose to 104.2^o F, the pulse became 110 to 120 and the respirations 30 per minute. Death occurred Aug. 21, 1931, at 9.50 A.M.

POSTMORTEM EXAMINATION

The essentials of the postmortem examination made an hour after death by Dr. Edwin F. Hirsch were: marked hyperplasia of the spleen (myelogenous leukemia); sarcoma of the left scapula; metastatic sarcoma of the left axillary lymph nodes, the biliary lymph nodes, the spleen, liver, pleura and twelfth thoracic vertebra;

marked calcified senile arteriosclerosis of the aorta and its main branches; moderate cloudy swelling and fatty change of the myocardium; senile arteriosclerotic atrophy of the kidneys; edema and hyperemia of the leptomeninges; slight hydropericardium, and organizing fibrinous perisplenitis.

The body weighed 113 pounds (50.4 kg.) and was 158 cm. long. Distal and lateral to the greater tubercle of the left humerus was a firm, freely movable, subcutaneous mass 2.5 cm. in diameter and elevated 2 cm. above the surrounding surface. This nodule was composed of dense white tissue lying close to the humerus between the two heads of the biceps brachii muscle. Tissues from this extended beneath the clavicle and dorsally to the subscapular fossa of the scapula, with which bone it was continuous. There was a marked destruction and roughening of the acromion process, spine, and glenoid fossa of the scapula. Continuous with this mass were firm, gray, enlarged axillary lymph nodes which likewise extended proximally into the lower left cervical lymph nodes. When completely dissected, the tumor tissue in the left axilla and anterior to the left scapula was roughly pyramidal in shape with the base 10 by 9 cm., resting on the left ribs and extending laterally 9.5 cm. The three faces of the pyramid were respectively 9 by 9.5 cm., 6 by 9.5 cm., and 8 by 9.5 cm. The surfaces made by cutting the tumor were mixtures of grayish pink and yellow necrotic tissues. The inguinal, right axillary and right cervical lymph nodes were not enlarged. The spleen measured 26 by 27 by 9.5 cm., and weighed 1620 gm. In and beneath the capsule on the diaphragmatic surface were many gray nodules with rounded surfaces; some as large as 2 cm. in diameter were in the firm, dark red, splenic parenchyma. The liver weighed 2170 gm. and had a smooth glistening capsule, except in the center of the right lobe where there was a glistening white nodule 4 by 2 mm. On surfaces made by cutting the liver there were many similar, small, indistinct white masses, and in the right lobe were four with diameters as much as 1 cm. Near the front and medial to the gall-bladder notch was a nodule 4 cm. in diameter with scalloped margins. On the right side of the body of the twelfth thoracic vertebra and continuous with the roughened bone was a nodule 1.7 by 1.5 by 1 cm. Deeply embedded in dense fibrous adhesions between the right lung and the diaphragm was a firm white nodule 1.2 by 1.3 cm. in its maximum dimensions.

The routine examination of the tissues included the lungs, innominate veins, superior and inferior venae cavae and their main branches, heart, aorta and its main branches, thoracic duct, thyroid gland, parathyroid glands, kidneys and ureters, pancreas, stomach, bowel, vagina, uterus, tubes, ovaries, urinary bladder, mesenteric lymph nodes, rectum, spine, ribs, and the bones.

MICROSCOPIC EXAMINATION

Blocks of the tissues for microscopic examination were fixed in Zenker's solution. Thin paraffin sections were stained with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin,¹ eosin-methylene blue, Azur II-eosin and hematoxylin as used by Maximow,² and anilin blue azan (Heidenhain's modification³). Other blocks were fixed in formalin, sectioned by the freezing method and stained for oxydase granules, according to Graham's benzidine technique (Mallory and Wright,¹ see page 524) and for lipins with Sudan III.

In the primary tumor, a scanty fibrillar stroma supports compactly and also loosely arranged spherical cells 10 to 20 microns in diameter with large oval or round vesicular nuclei (Fig. 1). The narrow margins of cytoplasm contain fine oxydase granules. Many of these cells are like the hemacytoblasts of Maximow.⁴ Elliptical cells 20 to 40 microns in diameter are intermingled with these. They have a scanty homogeneous or finely granular cytoplasm and two to six irregular vesicular nuclei. The distinction of nuclei is uncertain because of the marked lobulations. A few cells in mitosis are noted. Lipins are not present in their cytoplasm. These giant cells closely resemble megakaryocytes but differ in being smaller and in having larger vesicular nuclei and only a little cytoplasm. Many with few nuclei resemble the young megakaryocytes described and illustrated by Maximow⁴ (see pages 116 and 118). A third variety of cell, intermediate in size and 20 to 25 mm. in diameter, has indistinct margins and contains a round, oval or indented nucleus. There is no bone production.

The distended sinusoids of the hyperemic hepatic tissues contain many leukocytes in various stages of development, and a few erythrocytes. There are fatty changes of the cells toward the central portions of the lobules. The metastatic tumor tissues have a loosely

arranged and edematous fibrous stroma which supports round, oval or polyhedral cells 10 to 25 microns in diameter with granular cytoplasm and vesicular nuclei (Figs. 2 and 3a). There are many giant cells as much as 60 microns in diameter with five or more oval or indented nuclei. The nuclei of others are fused lobed masses. Many cells are in mitosis (Fig. 3, e, g, h). These giant cells are similar to those in the primary tumor except that they are larger, more frequently in mitosis, and often appear to be syncytial masses such as might have been made by a fusion of smaller tumor cells. There are transitional forms between mononucleated and multinucleated tumor cells (Fig. 3, a, b, c, e, f). The scanty cytoplasm contains oxydase and neutrophilic and eosinophilic Ehrlich granules (Fig. 3d). These tumor cells in the liver metastases vary more in size, shape and staining reaction than those in the primary growth (Fig. 2).

Most of the cells in the nodule in the region of the twelfth thoracic vertebra are 10 to 20 microns in diameter with round or elliptical vesicular nuclei. Each contains a single nucleolus, and is surrounded by a scanty homogeneous cytoplasm. Intermingled with them are oval or polyhedral cells 15 to 20 microns in diameter with basophilic, eccentric, elliptical or semilunar nuclei, sometimes multiple. Giant cells, similar to those described elsewhere, are scattered in the tissues.

Follicles are indistinguishable in the hyperplastic, lower left cervical lymph node tissues. The lymphoid cells (largely plasma cells) are in small groups and columns separated and intermingled with polynuclear leukocytes in various stages of development. Tumor cells, like those previously described, are focally disseminated among the lymphoid elements. In one region the fine fibrillar stroma is in interlacing bands that form alveoli filled with tumor cells. Neutrophilic and eosinophilic granules are demonstrated in the cytoplasm of the tumor and myeloid cells.

In a lymph node from the region of the left biceps brachii, the fine fibrous stroma supports a few lymphocytes and many myelocytes. The latter fill the sinusoids and largely displace the lymphocytes in the follicles. No tumor metastases are found.

In a lymph node from the region of the left adrenal gland there is a large amount of fibrous tissue in the capsule, septums and about the lymph nodules which are filled with plasma cells and lymphocytes.

Polynuclear leukocytes are scattered among young blood cells about twice as large as lymphocytes with colorless or faintly acidophilic cytoplasm and with blue, granular, band-shaped nuclei.

In a tracheobronchial lymph node plasma cells and occasionally lymphocytes are widely disseminated so that follicles are not distinguished. There are a few mononucleated or multinucleated giant cells, and slight fibrous changes.

The stroma of the spleen is loosely arranged in fine and coarse bands which support compact masses of myeloid cells and occasional groups of lymphocytes so dispersed that the malpighian corpuscles are not distinguished. The metastatic nodules of tumor tissue are in all respects similar to those of other secondary growths.

A nodule from the diaphragm is composed of tumor tissue resembling that previously described.

The bony trabeculae of the left humerus takes a pale stain. The fat cells, sinuses, and all other normal structures of the marrow are replaced by dense interlacing bands of fibrous tissue with various hemopoietic cells (Fig. 4). Erythrocytes are few and megakaryocytes are numerous. These latter cells are elongated with maximum diameters of 40 to 60 microns and have from fifteen to eighteen compact, strongly basophilic nuclei surrounded by scanty cytoplasm. Other di- and trinucleated spherical cells with diameters as great as 40 microns are occasionally seen, as are lymphocytes, plasma cells and polynuclear leukocytes. Of the granulocytes "band" cells predominate. Neutrophilic myelocytes are abundant, while eosinophilic or basophilic are rare.

In a vertebra the moderately increased fibrous stroma supports hemopoietic cells similar to those noted in the left humerus. Megakaryocytes are infrequent while mature polynuclear leukocytes are numerous. In sections from one block, certain marrow tissues are necrotic.

The dense connective tissue framework which predominates in preparations of the left humerus is practically absent in ribs. A fine reticular network envelops evenly disseminated marrow cells more nearly uniform in size and shape than those described above. Of these cells, those of the polynuclear series are most abundant and megakaryocytes are few. Fat cells, not noted in other preparations of bone, are present.

DISCUSSION

The clinical, hematological, and postmortem observations establish the diagnosis of chronic splenomyelogenous leukemia. The tumor of the left scapula is myeloblastic because it contains myeloblasts, myelocytes and megakaryocytes, and is malignant because of the metastatic growths in the spleen, liver, pleura and the axillary, biliary and cervical lymph nodes.

While sarcomas of the scapula are rare, no primary myeloid leukemia with sarcoma of this bone has been recorded. Fulghieri⁵ described myeloid leukemia associated, in the later stages, with a myeloblastic tumor of the femur. In his report of a sarcoma of the skull with secondary growths in the spine, Ribbert⁶ described cells like those of bone marrow, some of which contained hemoglobin. He considered them erythroblasts and was able to demonstrate transitional forms between erythrocytes and tumor cells. Christian,⁷ in his report of multiple myeloma, stated that the description of erythroblastic cells in Ribbert's case was inadequate and that the hemoglobin content might be only stain from surrounding erythrocytes. Borst⁸ mentioned malignant tumors with constituents like bone marrow in which occasionally he found all cells of the marrow but more commonly either megakaryocytes or leukocytes. Roman⁹ studied the tissues from two patients who had died with bone sarcomas. In one, with multiple malignant tumors of the bone marrow, there was extensive invasion of the spinal canal and cranial cavity and metastases into the retroperitoneal, inguinal, popliteal and mediastinal lymph nodes. Histologically he found lymphocytes, plasma cells, granulocytes, and megakaryocytes in the tissues. In the second tumor, a sarcoma of the skull, spinal column and long bones, there were widespread lymph node metastases. Microscopically myeloid cells were identified. He referred to the reports of Marchand, Gussenbauer-Chiari, Dittrich and Schmidt which, in some respects, were similar to those he described. Fischer,¹⁰ in his study of an endothelioma of the liver, observed cells like those of bone marrow, namely erythroblasts and myelocytes. In his differentiation between myeloid sarcoma (myeloma) and malignant giant cell sarcoma of bone Stewart¹¹ mentioned the rarity of islets of bone in the latter tumor and stressed the malignancy of the growth, in contradistinction to myeloma which he considered benign. Froboese¹²

likewise stated that myelomas are not malignant because metastases rarely, if ever, occur. This contention of Froboese was made after a study of a malignant growth of the sternum which contained myeloblasts, myelocytes, erythroblasts and erythrocytes with their intermediate forms. Although Wallgren ¹³ found many myelomas with secondary growths he considered myeloma benign. Mieremet ¹⁴ found megakaryocytes, erythroblasts, erythrocytes and various forms of polynuclear leukocytes in a tumor of the adrenal. Herscher and Thevenard ¹⁵ observed malignant myeloblastic sarcomas: two of the skull and one of the sternum. Megakaryocytes and myelocytes were noted in the tissues. Saleeby ¹⁶ reported two symmetrical tumor masses of hyperplastic bone marrow attached to the inner surfaces of the fifth ribs in a hemiplegic patient dying of bronchopneumonia. He briefly summarized the literature on heterotopia of bone marrow and recorded five cases not associated with either leukemia or pseudoleukemia. Warren ¹⁷ described an extensive retroperitoneal tumor simulating bone marrow and concluded that the hemacytoblast of Maximow was the predominant cell. In addition to the type cell, he found erythroblasts, erythrocytes, megakaryocytes and cells of the polymorphonuclear series. Hirsch ¹⁸ reported a myeloblastic sarcoma of the cranium with extensive local destruction and metastases to the cervical lymph nodes and right lung. He found myelocytes, leukocytes and megakaryocytes in the growth.

SUMMARY

Chronic splenomyelogenous leukemia complicated by a malignant tumor of the scapula, with metastases to the axillary, biliary and cervical lymph nodes, liver, pleura, spleen and twelfth thoracic vertebra, is described in this report. Both the primary and secondary tumors contained cells resembling components of the bone marrow.

I wish to express my appreciation to Mrs. Eleanor A. Hunt of the Department of Anatomy of the University of Alabama for the illustrations in Figure 3.

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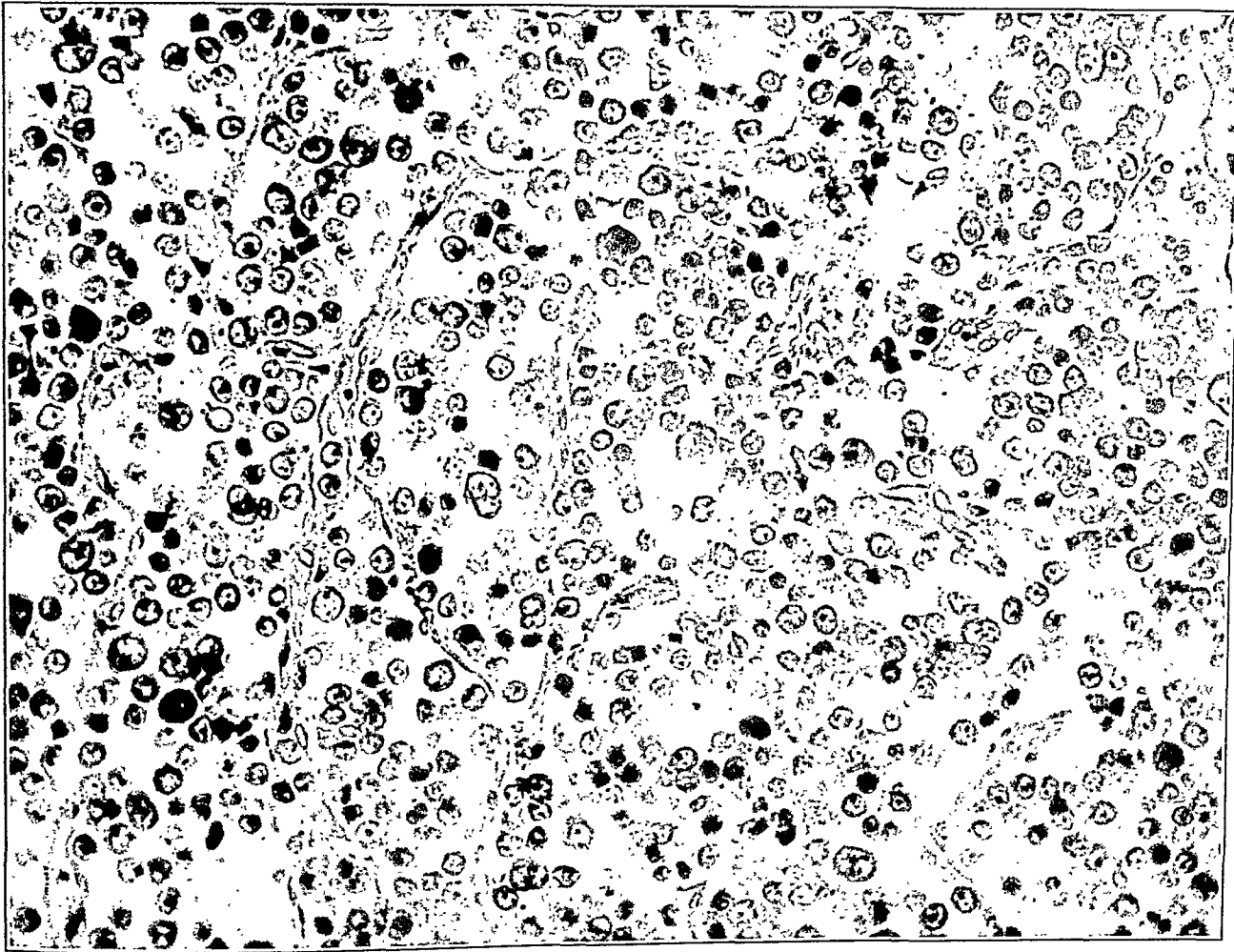
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DESCRIPTION OF PLATES

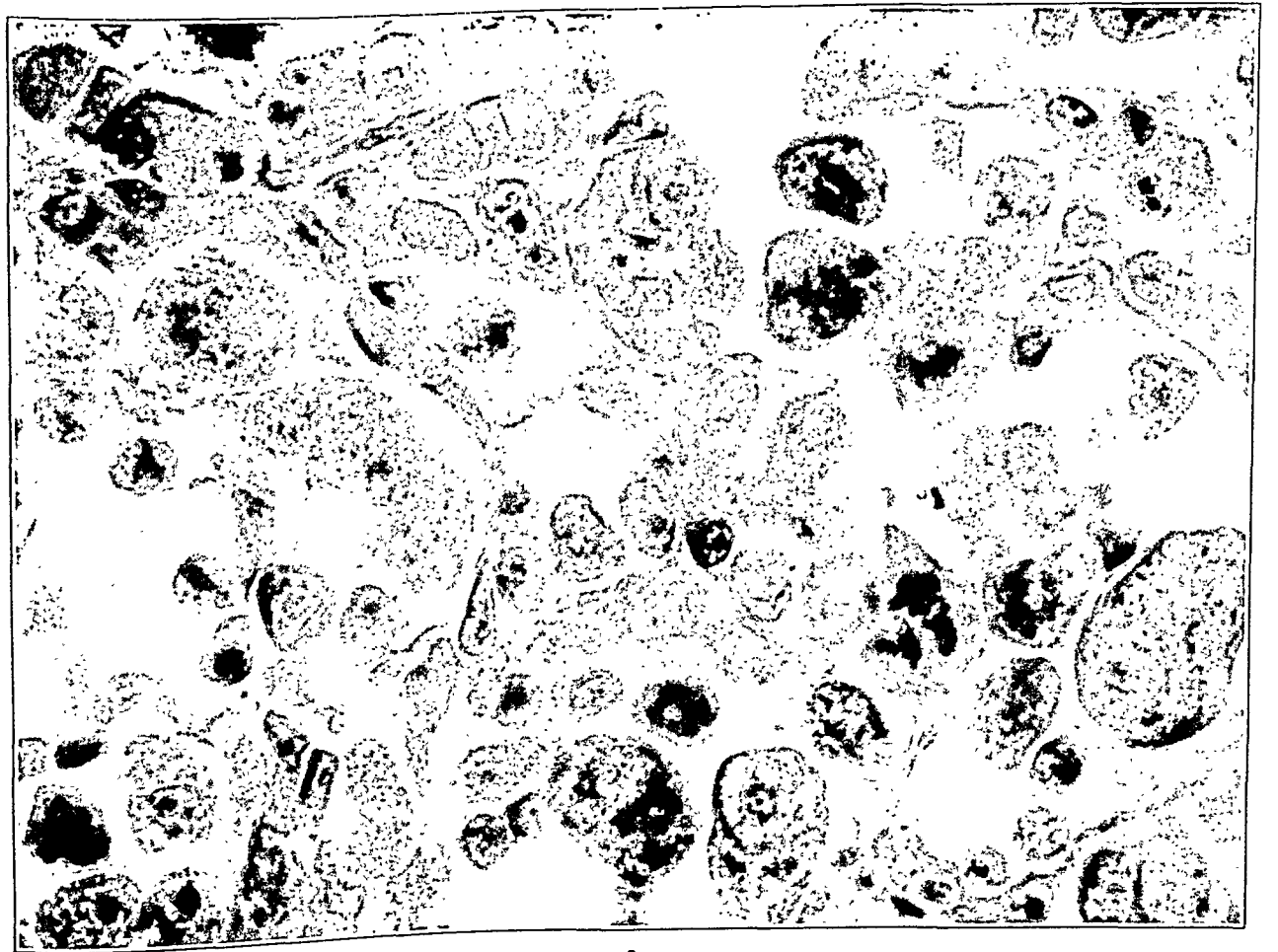
PLATE 20

FIG. 1. Photomicrograph of the primary tumor to illustrate general configuration of the tissue. $\times 290$.

FIG. 2. Photomicrograph to illustrate marked variation in size and shape of tumor cells in liver metastases. $\times 840$.



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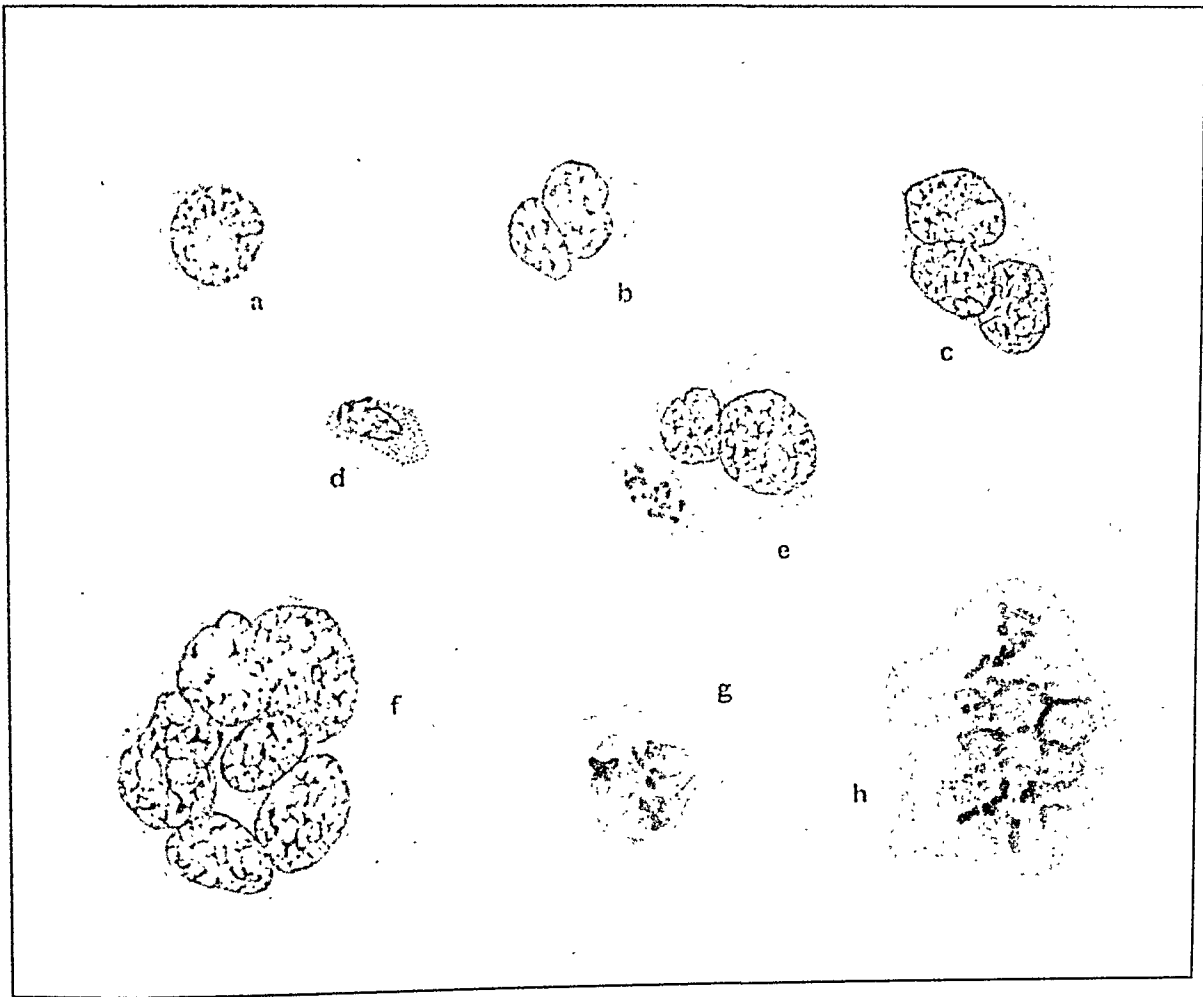
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PLATE 21

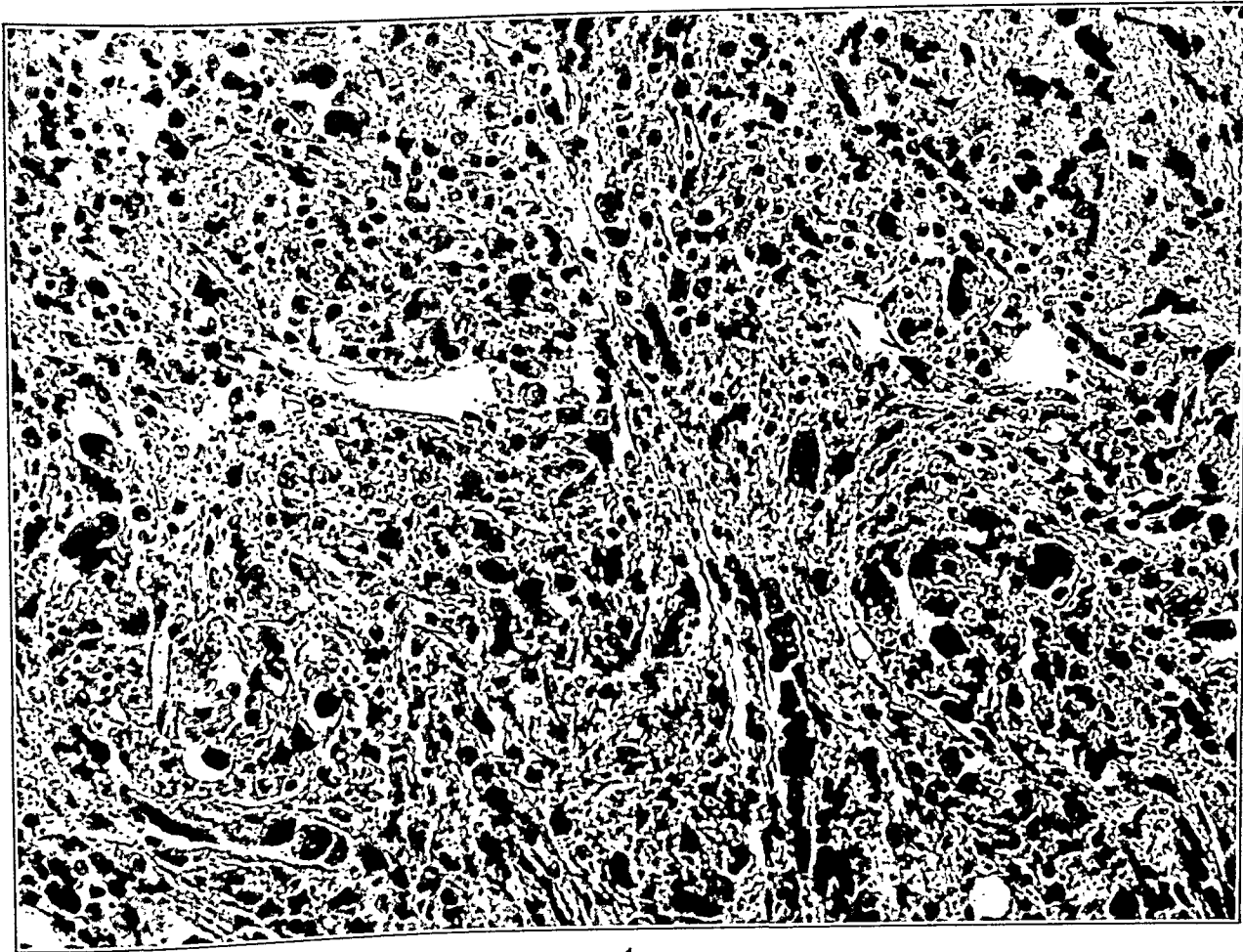
FIG. 3. Several types of tumor cells drawn at table level with a camera lucida. Approximately $\times 750$.

- a = Mononucleated tumor cell.
- b = Binucleated tumor cell.
- c = Trinucleated tumor cell.
- d = Tumor cell with Ehrlich granules in cytoplasm.
- e = Trinucleated tumor cells. Note one nucleus in mitosis.
- f = Large multinucleated cell of the megakaryocytic variety.
- g = Tumor cell in multipolar mitosis (metaphase).
- h = Large megakaryocytic type of tumor cell in multipolar mitosis (prophase).

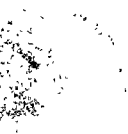
FIG. 4. Photomicrograph to illustrate interlacing bands of fibrous tissue and variety of hemopoietic cells in the marrow tissues of the left humerus. $\times 290$.



3



4



REPORT OF A CASE OF RETOTHELIO-SARCOMA (RETICULO-SARCOMA) OF THE CEREBRAL HEMISPHERE *

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As the tumor we are about to describe is of an almost unique character and presents considerable difficulties in the way of diagnosis, it seems desirable that the case should be reported in detail. Should one look through modern textbooks on brain tumors such as Cushing's¹ or Sachs's² one would find no description of an exactly similar neoplasm; nor have we been able to find any such detailed in shorter articles.

The specimen was removed at operation in two stages by Dr. B. N. Carter at the Cincinnati Children's Hospital, and submitted to our department for diagnosis. After a simple bone-flap decompression to relieve intracranial pressure, the tumor was later removed as completely as the condition of the patient allowed at a second operation. A third procedure, to extirpate what was left of the deeper extension of the growth, was not feasible. The tumor lay about 3 cm. beneath the cortex of the left temporoparietal region and extended deeply toward the central part of the brain, where it was infiltrating to such an extent that sharp dissection was necessitated. Otherwise, the tumor had a capsule which rendered enucleation relatively simple elsewhere. Dr. Carter reports that he explored the left ventricle and found its lining smooth and apparently unaffected by the growth.

REPORT OF CASE

Clinical History: The patient was a white girl, 9 years of age, who had been well up to about eight months ago. She was a normal, full-term delivered baby and, aside from having her tonsils removed a year prior to the onset of symptoms, had had no illnesses of consequence. The present attack began with severe frontal headaches, accompanied by vomiting of the projectile type, which came on about twice a week and were unaccompanied by nausea, usually following breakfast or supper. There was no abdominal pain. The child was bright and continued to go to school up to four weeks ago. She had had no disorders of

* Received for publication August 1, 1932.

taste, smell or hearing, but had a sudden attack of blindness following an X-ray examination about a month previous to admission to the hospital.

The essential findings on physical examination were as follows: The right pupil was larger than the left, the extraocular muscles acted normally, the optic discs were swollen and striated, their margins indistinct; the arteries of the fundi were contracted and their veins congested. There were no retinal hemorrhages. General physical examination was negative, save for the neurological findings. The knee jerks were absent and the Kernig, Babinski, Oppenheim and Romberg tests were negative.

During her stay in the hospital a right homonymous hemianopsia was determined and a diagnosis of left frontal tumor was made. After a month the Kernig sign became positive and the child's spleen was found to be palpable.

Following the operation there was paralysis in the right arm and leg, and ankle clonus on the right. The abdominal reflexes disappeared on that side also.

The patient died almost two months after the operation and, although every effort was made to obtain a permit for complete autopsy, permission was limited to an investigation of the brain. This was carried out by the hospital's pathologist, Dr. Irving H. Schroth, without any material addition to our knowledge of the case, as nothing was found except ventricular dilatation and foci of infection in the neighborhood of the operative site. It is greatly to be regretted that our case must, therefore, remain uncompleted.

THE TUMOR

Gross Examination: The specimen consisted of a mass 7.5 by 6.5 by 6 cm. in size, fairly well circumscribed and enclosed in a sort of capsule, excepting at one point where the surface was irregular and ragged. A few small tags of brain tissue adhered to the capsule. The section surface presented circumscribed, pearly white areas that alternated with serpigenous streaks or local spots of an orange-colored tissue that gave the tumor a xanthomatous appearance. The growth was rather nodular beneath its capsule and the pearly areas suggested cellular alveoli, while the orange material may have been necrotic tissue or a stroma with lipid infiltration.

Microscopic Examination: Routine hematoxylin-eosin sections at once revealed that we were dealing with a most unusual brain tumor. Instead of the compact picture of a glioma, a meningioma, or that of an ordinary vascular or ependymal tumor, one finds that the greater part of the sections examined are composed of nests of enormous and very bizarre giant cells and syncytia which lie in a stroma of rather edematous connective tissue (Fig. 1). The cells have a

most pleomorphic appearance, ranging through the gamut of morphological variation — they may be spheroidal, turnip-shaped, fusiform, unipolar or bipolar with long tail-like processes, or they may be cylindroid (Fig. 2). They contain a varying number of nuclei, which may be single and of enormous size, or multiple, in which case there may be from ten to twenty or more nuclei of varying size and shape. Some cells are strewn with minute vesicular nuclei. Mitotic figures are very abundant and range from quasinormal karyokinetic forms to the most bizarre and abnormal, multiple, asymmetrical, cross- or y-shaped types. It would be impossible to describe a nucleus typical for this growth, so varied are these in their morphology; on the whole, however, they show a deeply stained but delicate chromatin network and one or more distinct and often enormous nucleoli. The cytoplasm of most of these cells is pale and vacuolated, though it may be slightly denser in the cell processes where it has become more compact. Micrometer measurements show that the large cells approximate 50 to 80 microns in their longest diameter, although one of them has attained a size of 192 by 80 microns. Their nuclei, when single, may be about 20 microns in long diameter, but one of the largest observed measured 40. The stroma of the tumor gives the general impression of trabeculae, between which are sinusoids that contain the tumor cells; one immediately thinks of the splenic architecture when one first looks at this tumor under low power (Roussy and Oberling³).

While casting about for the possible origin of such cells, it is natural to search for fields where they are most uniform in their morphology, most free from necrosis or degeneration and apparently in their most healthy condition, as evidenced by more normal mitotic figures. Such areas may be found in this tumor and they exhibit small, actively dividing polygonal cells which tend to be joined through anastomotic processes — cells that remind one of the retothelium (reticulo-endothelium) of the lymphoid or myeloid tissues. On the average, they measure about 8 microns in diameter and consist mostly of a somewhat vesicular nucleus and a narrow rim of cytoplasm with radiating processes.

As this tumor came from the brain, it might be derived from a number of sources: its healthier areas indicate a retothelial origin, but against this is the marked lack of vessels and the site of the growth; for although ordinary vascular tumors may be encountered

anywhere in the organ, the retothelial type is notably limited to the base, medulla, cerebellum and the spinal cord (Cushing,¹ Sachs,² Roussy and Oberling,³ and Cushing and Bailey⁴). Other possible origins might be: (a) neuroglia, (b) ependyma, (c) meninges, (d) true nerve cells or ganglion cells. A metastatic origin cannot be excluded, but there is nothing in the history or physical examination to indicate another primary focus, unless it be the spleen or the tonsils. The spleen was not noted as enlarged until some time after admission. Concerning the tonsils we have no available pathological data, but they were not stated to be involved by neoplasia.

SPECIAL METHODS OF EXAMINATION

Neuroglia: We undertook impregnations of neuroglia cells and fibrils by means of a modified del Río-Hortega method on frozen sections (Foot,⁵ and the new Wilder method on paraffin sections⁶); neuroglia fibrils were also sought with the familiar and reliable phosphotungstic acid hematoxylin stain of Mallory. These methods fail utterly to demonstrate any neuroglial elements whatsoever; the large cells do not impregnate black, as should derivatives of spongioblasts, but become either yellow or old gold. Their processes, it is true, are sometimes blackened, but not invariably, and they are never attached to vessel walls but trail off into the stroma.

Ependyma: The preceding methods should demonstrate ependymal cells as well as neuroglia, but they fail to do so. In the phosphotungstic acid hematoxylin sections one occasionally finds groups of tiny, violet granules clumped at the center of the cell, not far from its nucleus. Although this suggests the presence of blepharoplasts, it can scarcely be taken alone as an indication of ependymal origin in the face of the negative findings with neuroglial silver impregnations. Furthermore, the tumor does not obviously involve the wall of a ventricle.

Meninges: Meningeal origin, we think, may be ruled out on the basis of the site of the tumor and of its architecture, which in no way suggests that of a meningioma.

Neurocytes and Ganglion Cells: Here we encounter difficulties: sections impregnated by a modified silver nitrate method (Foot⁷) (Fig. 1), and by the Rogers method⁸ show that the large cells may closely simulate ganglion cells; they have their general outline, their proc-

esses impregnate like the dendrites of pyramidal cells; and strange distorted forms resembling unipolar and bipolar neuroblasts may be found as gigantic cells. There are no demonstrable nerve fibers, however, and although one might construe some of the smaller cells that surround their larger fellows as being capsule, or satellite cells, this would require rather too much imagination, for such an arrangement is only occasionally found in this tumor. The cells lack Nissl bodies, which are well brought out by the silver nitrate method in normal nerve cells. The Rogers method, which picks out medullated and non-medullated fibers with precision, as a rule fails to demonstrate any here. The silver methods do impregnate the tails and processes of the fusiform and polar varieties of the tumor cells, sometimes giving them the appearance of coarse fibers, but this is also noted in the phosphotungstic acid hematoxylin sections where they take on a deep blue stain occasionally.

Retothelial Cells: We are adopting the term "retothelial" for reticuloendothelial cells, as suggested by Roulet⁹ in his paper on "Retothelsarkom." He says: "The term 'Retothelsarkom' is not only shorter than 'Reticuloendothelsarkom,' but it is likewise more correct, as the tumor takes its origin not from the *endothelium* of the lymph sinus, but from the covering cells of the reticular framework, which are better termed '*retothelial*' cells." This seems logical to us. Having considered the other possible origins of our tumor we are thrown back upon the first hypothesis of retothelial origin: let us test its validity in this case. Silver impregnation by the Foot and Foot method (Variant 3),¹⁰ which is excellent for connective tissue, demonstrates the presence of abundant collagenous fibers in the trabecular, hyaline portion of the stroma; from this black reticulum fibers radiate into the cell nests. Where the tumor cells are large and atypical the reticulum seldom penetrates between them, although it may do so occasionally. The picture is somewhat like that seen in a carcinoma. Where the cells are smaller and more uniform in their morphology, in the areas already cited as probably constituting the most recent and "normal" tumor growth, the reticulum forms a rather dense network among the cells, running along their cytoplasmic processes and involving them in a loose felting of black fibers (Fig. 3). In these sections the resemblance to splenic tissue is much heightened by the presence of abundant collagen and some reticulum in the trabeculoid stroma.

Roussy and Oberling describe an "infiltration lipo-lipoidique" in their "angioréticulomes" and compare it to the infiltration seen in such dyscrasias as Pick-Niemann's disease, believing it to be a process expressing necrosis of brain tissue, setting free of lipid substances from this and subsequent phagocytosis of this fatty material by the tumor cells. Frozen sections of our tumor, stained with scarlet R and hematoxylin, show a very rich lipid content. Fine droplets form a sort of "milky way" in the hyaline portions of the stroma; there are collections of coarse drops and masses of this material at the periphery of necrotic areas and, most important, large numbers of droplets may be demonstrated in the tumor cells themselves (Fig. 4). These may be completely filled with lipid material, so that they take on the familiar "mulberry" appearance — the cell body a vermilion mass, the blue nucleus embedded in closely packed red droplets which may be found in the cell processes — just as one sees melanin granules in those of melanoblasts. Other cells may show a few small lipid droplets. A discussion of the lipid content of the neuroglia may be found in a recent article by Sjövall.¹¹ The author cites Roussy and Oberling among other observers of this phenomenon. As the fat in the macroglia is deposited in smaller amounts than in the tumor cells under discussion and as Roussy and Oberling were cognizant of this when they mentioned it as a criterion of mesenchymal origin in the case of retothelial tumors, we may dismiss this as having but relatively slight importance in this case.

DISCUSSION

With these data at our disposal we cannot seriously consider a neurogenic origin for this tumor, however alluring may be the apparent similarity of its larger cells to ganglion cells; all our evidence points more clearly to the retothelium. That these monstrous cells may be of retothelial origin is indicated by past experience (Foot¹²) as well as by comparison with more recent articles on the subject, such as Roulet's. Against this diagnosis stands the dictum that such tumors occur in the base, hind brain or cord, rather than in the hemispheres; there seems to be no valid reason why they should not be found there as well; it is probable that they merely do not happen to have been met with in that situation. This tumor differs from the cerebral retotheliomas hitherto described in its very active growth

and definitely sarcomatous nature. We have recently seen and studied one of the type described by Cushing and Bailey and by Roussy and Oberlin, which was located in the pontine region and corresponded exactly with their descriptions. In the case of that tumor there are abundant blood vessels and channels, but the mitoses and the very marked and bizarre distortions of morphology noted in our case are both absent. There are, it is true, numerous syncytial giant cells, but these are of a more orderly type of growth and exhibit few of the vagaries of those in the tumor we are discussing.

Authorities agree that these neoplasms originate in perivascular or intervascular congeries of retothelial cells, and the fact that they may be relatively poorly supplied with vessels is also recognized. It would appear that our case represents a malignant metamorphosis of such a tumor — Cushing and Bailey's "hemangioblastoma," or Roussy and Oberling's "angioréticulome." It would, under this hypothesis, constitute a retotheliosarcoma, or reticulosarcoma under the old terminology. One should avoid the use of the term "endothelioma" in speaking of such a tumor in order to escape the confusion that might arise between it and an "endothelioma of the dura." Many of the tumors described by Roulet as occurring in lymphoid tissue are so strikingly similar to ours in their appearance, that his Figs. 1, 2, 5, 6, 10 and 11 might have been taken from fields in our sections. There is no record of lymph node enlargement anywhere in our case and the spleen has only recently become palpable, which might be explained on the basis of a simple toxic splenitis. The chance of the tumor's arising in that organ seems to be remote, in view of the history and of the large size of the brain tumor before splenic enlargement was apparent.

SUMMARY

A large tumor is reported, occurring in the left cerebral hemisphere of a 9 year old girl. The growth is described in detail and seems to have originated in the perivascular and intervascular retothelium. It differs from tumors hitherto described in its site, its manifestly malignant nature and more marked pleomorphism. Such tumors usually occur in the base of the brain, the hind brain, or the spinal cord; this one was deeply embedded in the left temporo-parietal region. It shows extremely abundant mitoses and equally

marked variation in the size, shape and nature of its cells. It contains an abundant reticulum and its cells show marked lipoid infiltration of the "xanthomatous type." We believe that we have ruled out nervous or neuroglial origin by means of specific methods of impregnation and staining.

There is no evidence of a primary tumor elsewhere in the body, as determined by physical examination, and the symptoms in the case have been exclusively referable to intracranial pressure, no other symptoms having been observed in an otherwise healthy and normal child.

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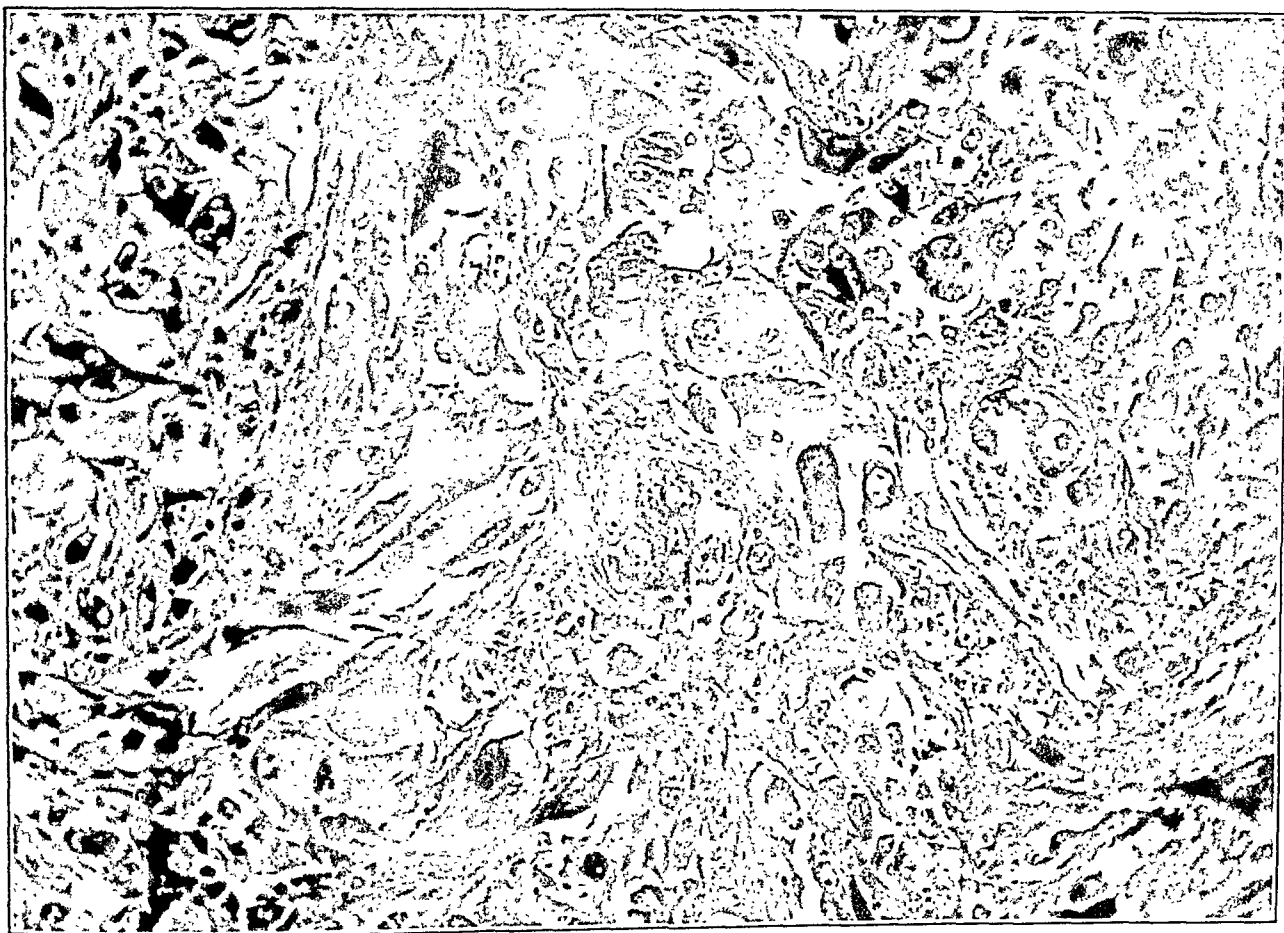
DESCRIPTION OF PLATES

The photomicrographs were all taken by Prof. J. B. Homan of our Department of Medical Art, with the assistance of the author.

PLATE 22

FIG. 1. Low power view of a field impregnated with silver nitrate. This shows the cell morphology and demonstrates the arrangement of the supporting tissue. $\times 200$.

FIG. 2. High power view of a group of giant cells stained with phosphotungstic acid hematoxylin. Notice the clusters of small nuclei in some of the syncytia (8 microns in diameter). The large cell at the center, with a necrobiotic nucleus, measures 128 microns in length, the large nucleus just below it 32 microns and the darker nucleus below this 24 microns. $\times 800$.



I

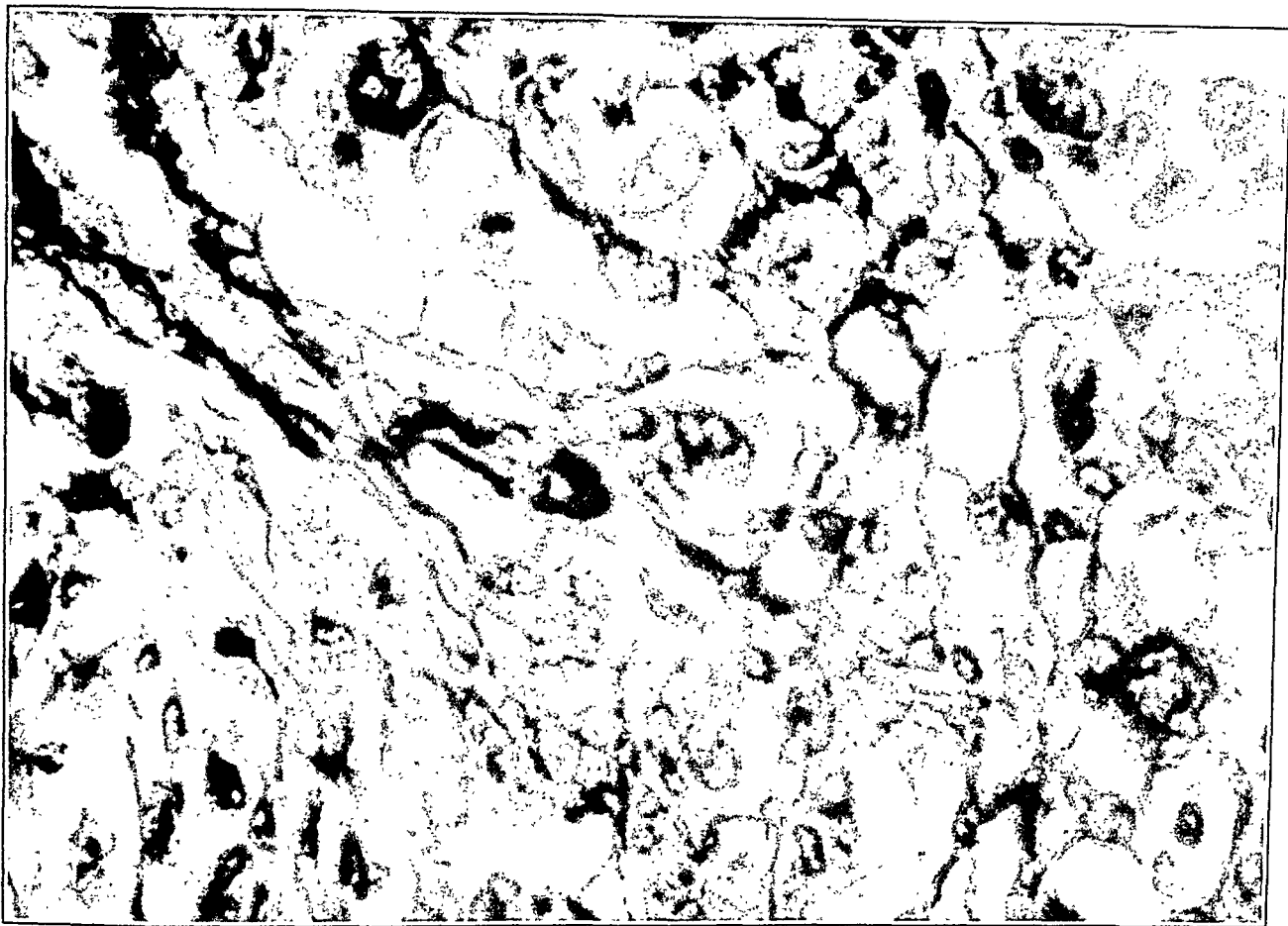


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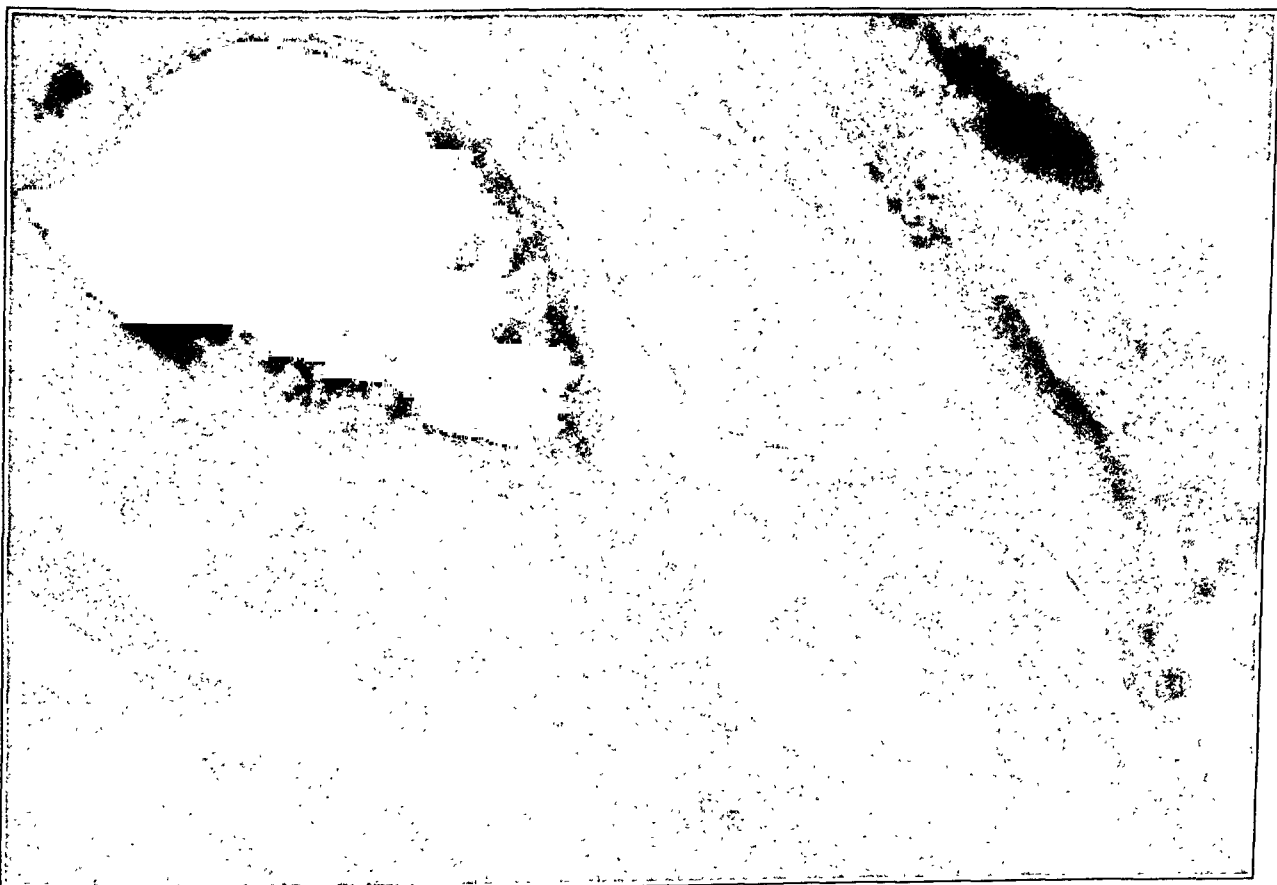
PLATE 23

FIG. 3. High power picture to show the reticulum in a section (Foot and Foot impregnation). One mitotic figure is present. The cells are more uniform in size and shape and resemble retothelial cells more closely than do the syncytia shown in the other figures. $\times 800$.

FIG. 4. High power field from a frozen section stained with scarlet R and hematoxylin and photographed with a blue filter that makes the cellular detail insignificant while it emphasizes the lipoid droplets. The large cell, heavily laden with fatty material, measures 92 microns in its long diameter. $\times 800$.



3



4



EXPERIMENTAL STUDY OF THE EFFECTS OF POTASSIUM BICHROMATE ON THE MONKEY'S KIDNEY *

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Of the various metallic salts which have been most commonly employed as nephrotoxins experimentally, potassium bichromate stands as a rather poor third, being preceded by uranium nitrate and mercuric chloride. Undoubtedly there are good reasons for its lack of popularity but these are not often mentioned in the writings. From certain remarks in the literature one suspects that the chief objections to chromium compounds are the marked local irritative and corrosive properties and the violent gastro-intestinal manifestations. When administered subcutaneously compounds of chromium are quite likely to cause severe necrosis and sloughing at the point of inoculation, even though comparatively dilute solutions are employed. The same is of course true for corrosive sublimate. With both chromates and sublimate it is difficult to obtain constant results so far as the kidney is concerned because much of the poison may be held up at the point of entrance into the body in one animal, while in another quite the opposite may be the case. Ophüls¹ long ago recognized the irritative effect of potassium chromate and sought unsuccessfully to overcome it by mixing with the solution an equal amount of sodium carbonate. In a previous communication Ophüls² had observed the inconstancy of tubular necrosis in the kidneys of rabbits receiving quite large amounts of potassium bichromate.

The chromates have almost universally been regarded as pure tubular poisons, in contrast to uranium and mercury compounds which admittedly give certain evidences of damage to the glomeruli. Among the earlier descriptions of the renal lesions produced by chromic compounds that of Thorel³ stands out because of his recognition, careful description and excellent illustration of the regenerative process in the affected tubules.

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There is rather general agreement in the literature that the chromates, like other metallic poisons, affect the proximal convoluted tubules predominantly or wholly. In his great work on the morphology of kidney secretion Suzuki ⁴ found that potassium bichromate damaged primarily the first and middle divisions of the proximal convoluted tubules, although if larger doses were administered the more distal part of the tubule was affected as well. Later Oliver ⁵ and still more recently Jessen ⁶ have confirmed Suzuki's observations in this respect.

As with uranium, practically all that is known about the renal manifestations of chromic compounds has been gained from experimental studies. In one of the earliest contributions in this field Kabierske ⁷ mentions the common employment of chromium as a tissue caustic in human beings, and instances of severe poisoning resulting therefrom. Major ⁸ has recorded a case of fatal chromic acid poisoning occurring in a man suffering from cancer of the cheek, treated after curettage with crystals of this substance. In view of the great rarity of such accidents it may be of interest to list some of the more salient clinical features of this case. The next day after application of the acid, the urine, which had previously been normal, contained 4 gm. of albumin per liter. Three days later the quantity had increased to 7.30 gm. per liter with hyaline and granular casts, red blood cells and numerous degenerated epithelial cells. After about forty-eight hours an almost complete anuria developed, but later the urine output was high and of low specific gravity. Death occurred on the twenty-ninth day, the blood urea at this time being 340 mg. and the creatinin 17 mg. per 100 cc. of blood. A definite acidosis developed but yielded readily to alkali therapy. At autopsy the kidneys presented a mottled appearance with the cortex swollen and the glomeruli very indistinct. There was no ascites or anasarca. Microscopic examination disclosed extensive destruction of the tubular epithelium with débris in the lumen and great swelling of the intact epithelial cells, especially in the convoluted tubules. Although a few mitotic figures were present in the cells of the affected tubules evidences of regeneration were not striking. For the most part the glomeruli appeared normal and in general the microscopic appearance was that of a pure tubular nephritis. Bernard ⁹ quite recently has described the clinical aspects of two instances of chromate poisoning in the human, one from his own practice, the other that of

Jonsson.¹⁰ Both patients recovered after a stormy illness during which severe gastro-intestinal disturbances, characterized by vomiting and diarrhea, were as prominent a part of the clinical picture as the kidney disease. In each instance the poison had been taken by mouth.

MacNider¹¹ in a general review of acute experimental nephritis has well summarized the general opinion concerning the acute effects of chromate upon the kidney in the following words:

"1. The chromates induce a renal injury which is primarily tubular and affects in particular the epithelium of the convoluted tubules. Late in such a nephritis the glomeruli participate in the injury.

2. Secretory diuretics such as caffein, theocin, and theobromine sodium-salicylate are of no value and may be injurious.

3. The chromates lead to a rapid reduction in urine formation, an inability of the kidney to eliminate phenolsulphonaphthalein, and a retention of chlorides, blood urea, non-protein nitrogen and creatinin.

4. The chromate nephritis is associated with the development of an acid intoxication which is likely a retention phenomenon and which is not favorably influenced by the use of an alkali."

EXPERIMENTAL METHODS

Our interest in potassium bichromate as a nephrotoxin was aroused by the statements of Suzuki and others to the effect that it exhibits a selective affinity for a different division of the proximal convoluted tubule than uranium and sublimate, salts previously employed by Hunter^{12, 13} in an investigation dealing with acquired resistance of regenerated tubular epithelium to metallic salts.

In view of Suzuki's statement that regenerated tubule cells after chromate poisoning were less resistant to subsequent and increased injections of the same poison we wished to determine if possible why this should occur with chromium compounds, whereas the opposite is true after injury by uranium and mercury. It appeared also that if potassium bichromate damages the end of the proximal convoluted tubule opposite to that injured by uranium such a localization would afford opportunity of observing the mode of epithelial regeneration and perhaps solve the problem as to the origin of the atypical flat-

tened cells, which in our experience were always found after poisoning with uranium and mercury.

Having available kidney material from normal monkeys and six living animals (*Macacus rhesus*) it was decided to utilize them for the problem after some rather disappointing preliminary experiments with rabbits. No attempt was made in either rabbits or monkeys to control the acidosis known to result from acute chromate poisoning, because we wished to produce as marked kidney damage as possible.

The essential data relating to dosage, duration of the experiments, clinical course and fate are summarized in Table I, but since the number of monkeys in the series is small it may be permissible to describe the findings in each case separately.

Monkey 1

Preliminary experiments with rabbits had shown that 1 cc. of a 2 per cent aqueous solution (0.02 gm.) per kilogram of body weight administered subcutaneously was near the lethal dose of potassium bichromate for this animal. Lacking available data as to the amount required to kill a monkey the first animal was given the same quantity per kilo as had been used for rabbits (36.3 cc. containing 0.726 gm. $K_2Cr_4O_7$). The rapidly fatal termination indicated that the dosage was far too great and that if subsequent experiments were to succeed the quantity must be decreased appreciably (Table I).

Macroscopically the kidneys displayed no changes.

Microscopic Examination: The epithelium of the entire proximal convoluted tubule stains faintly with eosin. Under high power the finer structure of the cytoplasm appears quite indistinct, the granules rarely being plainly outlined. The nuclei are unchanged. Only occasional cells show actual necrosis and desquamation. The lumina of many tubules contain a flocculent bluish substance but no distinct cast formation is noted in any of the tubules. The intertubular vessels of the cortex and medulla are almost uniformly engorged with blood. The glomerular capillaries, however, are frequently devoid of blood and it is rare to find more than two or three distended loops in any one glomerulus. The glomeruli for the most part do not fill the capsular space, which, aside from the presence of a few erythrocytes in a number of instances, contains no stainable material. In sections stained with hematoxylin and eosin it is difficult to distinguish

TABLE I
Potassium Bichromate Experiments with Monkeys

Monkey No.	Dates of injection	Duration of experiment	Site of injection	Amount	Clinical course	Fate
M-1	2/17/30	Less than one day	Subcutaneous	36.3 cc. 2 % Sol.	Injected 5 P.M. Found dead 7 A.M. on 2/18/30	Died
M-2	2/18/30	Less than one day	Subcutaneous	10 cc. 2 % Sol.	Injected 5 P.M. on 2/18/30 Found dead 7 A.M. on 2/19/30	Died
M-3	2/19/30 3/27/30 4/17/30 5/ 1/30	71 days	Subcutaneous " "	2 cc. 2 % Sol. 2 cc. 2 % " 4 cc. 2 % " 6 cc. 2 % "	Nausea, vomiting and anorexia for one day after each injection	Died during the night of 5/1/30
M-4	6/30/30 7/21/30	22 days	Subcutaneous "	1 cc. 2 % Sol. 2 cc. 2 % "	Same as M-3	Died 12 hours after last injection
M-5	6/30/30 7/21/30 8/18/30 10/13/30 12/ 6/30	160 days	Subcutaneous " " "	1 cc. 2 % Sol. 2 cc. 2 % " 4 cc. 2 % " 4 cc. 2 % " 5 cc. 2 % "	Same as M-3	Died about 20 hours later
M-6	6/30/30 7/21/30 8/18/30 10/13/30 12/ 6/30	163 days	Subcutaneous " " "	1 cc. 2 % Sol. 2 cc. 2 % " 4 cc. 2 % " 4 cc. 2 % " 5 cc. 2 % "	Same as M-3	Killed 12/9/30

glomerular epithelial and endothelial cells because of the indistinctness of the basement membrane. The majority of the glomerular nuclei stain almost black with hematoxylin, indicating pyknosis. Even with the aid of the azocarmine stain it is not always possible to determine whether the pyknotic nuclei belong to epithelial or endothelial cells, but it appears that both are affected. The glomerular basement membrane stains faintly in comparison to that of the tubules. It does not appear to be split or beaded. Numerous orange-colored, droplet-like bodies of various sizes lie in the glomerular epithelial cells and capillary basement membrane.

Monkey 2

This animal received a single injection of 10 cc. of 2 per cent potassium bichromate (0.2 gm.); death occurred within 14 hours (Table I). The kidneys appeared normal grossly, but in microscopic sections there is evidence of damage to the epithelium of the entire proximal convoluted tubule, equally severe in all of the several divisions. The cell nuclei are condensed, small, deeply stained and not infrequently broken into granular bits, while the cytoplasm is swollen, filled with tiny vacuoles and narrows the lumen, in which there are frequently homogeneous hyalin-like eosinophilic masses varying in diameter but often about the size of an erythrocyte. The cells lining the other tubules are intact and unchanged with coarsely granular, acidophilic material present in their lumina, particularly the collecting ducts.

The glomeruli more nearly fill the capsular space than in Monkey 1, by reason of a greater blood content. The capsular spaces are empty. Azocarmine preparations reveal a diminution of both glomerular epithelium and endothelium, with a poorly stained, frayed and beaded capillary basement membrane. Droplets like those described in Monkey 1 are again numerous.

Monkey 3

Over a period of 71 days the monkey received four injections of potassium bichromate, totalling 14 cc. (0.28 gm.) Death came soon after the final administration of the drug. Further details of the experiment will be found in Table I.

The gross appearance of the kidneys was normal.

Microscopic Examination: Sections stained with hematoxylin and eosin disclose coagulative necrosis of the epithelium throughout the length of practically all proximal convoluted tubules. The degree of necrosis varies but generally is complete, often with a loss of individual cell outline. Nuclear pyknosis and karyorrhexis is evident everywhere in the involved tubules and all divisions of the proximal convoluted tubules appear to be equally affected. Much of the epithelium lining the other tubular units has desquamated, apparently as a result of postmortem changes. In the labyrinths the nuclei of the tubular cells stain unusually deeply with hematoxylin while the cytoplasm is distinctly acidophilic. About a few glomeruli are groups of relatively small and somewhat compressed tubules lined by flattened or low cuboidal epithelium with basophilic cytoplasm, representing segments of proximal convoluted tubules affected by earlier injections in which regeneration has taken place. Such tubules are encompassed by an abundance of connective tissue. The collecting tubules contain granular epithelial casts with an occasional pyknotic nucleus still present. Hyaline casts occur in certain of the collecting ducts. There is little blood in either the intertubular or glomerular vessels. The capsular spaces are large and empty. The majority of glomerular epithelial and endothelial nuclei are small and heavily stained. No apparent alterations are observed in the capillary basement membrane.

Monkey 4

As will be seen in the table this animal succumbed 12 hours following a dose of but 2 cc. (0.02 gm.) of potassium bichromate, after having recovered from the effects of a previous injection of 1 cc. of the same substance.

At autopsy the kidneys appeared normal.

Microscopic Examination: Sections reveal an intense swelling of the cells lining the entire proximal convoluted tubule, as evidenced by the increased granularity and pronounced acidophilic character of the cytoplasm. The nuclei show little change except beginning chromatolysis in some instances. Oftentimes the swelling is sufficient to close the lumina of affected tubules. In three places the origin of proximal convoluted tubules is made out and it can be demonstrated that the cells here are damaged in the same way and to the same ex-

tent as those farther along the tubule. Regenerative activity is not in evidence. None of the tubules contain casts.

The glomeruli are of average size and contain a moderate amount of blood. An increase of nuclei in the tufts and of the cells forming Bowman's membrane is present in all glomeruli with capillaries open sufficiently to permit a cell count. In addition the azocarmine stain demonstrates slight fraying and beading of the capillary basement membrane.

Monkey 5

Starting with an initial dose of 1 cc. of potassium bichromate the animal received altogether five injections (total 0.32 gm.), dying about 20 hours following the final administration and 160 days after the beginning of the experiment (see Table I for further details).

Macroscopically the kidneys are of average size, but on stripping the capsules there are seen on the cortical surface several small and shallow pit-like depressions.

Microscopic Examination: All blood vessels, including the glomerular capillaries, are fairly well filled. In the cortex there is evidence of very grave injury of the cells lining the convoluted tubules, distal as well as proximal. Although a good many such cells preserve their outlines well and retain their nuclei very few fail to show increased acidophilia of the cytoplasm and varying degrees of nuclear chromatolysis. The more frankly damaged cells exhibit coagulation of the cytoplasm and extreme condensation of the nuclei. Many are either in the process of desquamation or have already become loosened and lie free in the lumina. In a great many convoluted tubules cellular necrosis is complete with granular, pinkish detritus and fragments of nuclei lying inside the denuded and barren basement membrane, external to which are elongated, deeply staining nuclei of the interstitial connective tissue (Fig. 1). As yet there is no sign of regeneration of epithelium in the affected tubules, which is readily accounted for by the fact that the animal died less than one day following the last injection. Histologically it seems rather clear that only epithelium of the original and normal type has been affected by the nephrotoxin, since there are many tubules, which from their position in the labyrinths and proximity to glomeruli must be of the proximal and distal convoluted type, covered completely by an entirely different type of cell displaying no histological evidence of

injury. These tubules vary greatly in size and shape, are often small, irregular and tortuous, less frequently large and dilated. Always the lining epithelium is atypical for this location in that the cytoplasm is invariably basophilic and the cells are nearly all either flattened or low cuboidal. The nuclei are generally oval with the long diameter in the direction opposite that of the tubule, and contain a single large and prominent nucleolus. Individual cell borders are often obscure, suggesting a syncytium. Patency of the tubules is indicated by the presence of necrotic cells within their lumina. In places the new cells have grown over and enclosed bits of necrotic cellular débris staining brilliantly with eosin, in marked contrast to the bluish living cytoplasm about them. Rarely they are taller than normal, in palisade arrangement or heaped up in mounds. Calcification is lacking. Rarely a nucleus is observed in the process of mitotic division. In areas where entire groups of convoluted tubules have been relined by regenerated epithelium there is a distinct increase of interstitial tissue with slight lymphocytic infiltration. Henle's loop and the collecting ducts are filled with casts, mostly hyaline but sometimes vacuolated and apparently fatty.

The glomeruli fill the capsular spaces fairly well. Often the capsular epithelium seems to be increased but does not form epithelial crescents. In three instances erythrocytes are found in the capsular space. Under oil immersion a count of five glomeruli reveals 60, 59, 71, 56 and 73 capillary epithelial cells, a greater number than in any other animal of the series. The endothelial cells number 16, 16, 18, 16 and 18 in the same glomeruli. Their nuclei appear to be pyknotic. The basement membrane is slightly but definitely split and beaded. Coarse and fine hyaline droplets are numerous.

Monkey 6

The duration of this experiment was 163 days, the animal being killed 3 days after the final subcutaneous injection of 5 cc. of 2 per cent potassium bichromate (0.32 gm. total). In Table I will be found the amounts of the previous doses.

The gross appearance of the kidneys is quite like that described for Monkey 5.

Microscopic Examination: Microscopically there is evidence that the last dose of bichromate found little vulnerable epithelium on which to act, for nearly all convoluted tubules, both promixal and

distal, are found to be fully covered by cells of the regenerated type and only a comparatively few have been stripped bare of epithelium (Fig. 2). These show the usual picture of pyknosis and karyolysis of nuclei, coagulation of the cytoplasm and detachment from the membrana propria. The great majority of convoluted tubules are lined by atypical epithelium and are of all sizes and shapes, but tend to have large and dilated lumina almost invariably filled with granular, pinkish-staining débris. Oftentimes bits of such débris, and occasionally an entire necrotic cell with a shrunken nucleus, are found to have been overgrown by the proliferating new cells. Many of the regenerated cells are elongated, flattened and show little or no tendency to individuality. Frequently they are cuboidal and some even approach the normal morphology with spherical nuclei located near the base. Always, however, the cytoplasm is bluish. In some instances the basophilia is faint, but when such cells are examined under higher magnification it is found that the pinkish tinge is due to the presence of fragments of original epithelium enclosed by the new epithelium. Not infrequently regeneration has been excessive, the cells piling up in layers and partially or completely occluding the lumina. Mitoses are numerous and the majority of the nuclei are hyperchromatic (Fig. 3). There are numerous hyaline and fatty casts in the collecting ducts. Several sections include the glomerular end of proximal convoluted tubules and in all of these the lining cells are of the atypical flat or cubical regenerated variety. The interstitial tissue is slightly increased. The intertubular vessels contain a normal amount of blood. Although the capsular epithelium is increased in occasional glomeruli this is not striking or generalized. A few of the subcapsular spaces contain erythrocytes (Fig. 4). Capillary epithelial cells are more numerous than in the normal control kidney but are fewer than in the glomeruli of Monkey 5. Otherwise these cells are unchanged, but the endothelial nuclei stain deeply. The basement membrane of the glomerular capillaries is thickened and frayed. "Hyaline droplet" degeneration is well marked.

DISCUSSION

From the foregoing descriptions it is quite evident that the kidneys of Monkeys 1 to 4 exhibit only acute lesions, Monkey 5 both acute and chronic damage, and that Monkey 6 is the only animal in which a truly chronic stage of renal disease was obtained.

The effect of potassium bichromate in the dosages employed is not restricted to the two segments of the proximal convoluted tubule nearest the glomerulus, but on the other hand is clearly diffuse throughout the length of the tubule. The effect upon the tubular cells is quite like that of uranium nitrate and mercuric chloride. Early there is swelling, vacuolization and increased acidophilia of the cytoplasm and either pyknosis, karyolysis or karyorrhexis of the nuclei. As necrosis becomes complete many cells lose their connection with the basement membrane, fall into the lumen, and with others form loose granular masses which become more compact and hyaline-like as the casts pass through the loop of Henle.

The irregularity of absorption of bichromate after subcutaneous injection is well illustrated by the difference in the renal lesions of Monkeys 5 and 6, receiving like quantities of poison over the same number of days. In the kidney of Monkey 5 the comparatively few tubular segments with regenerated cells indicates that up to the time of the final dose not a great deal of tubular epithelium had been destroyed. The animal died about twenty hours following the last injection. Previous experience with uranium and mercury had shown that regenerative activity cannot be expected before four to five days after administration of these substances and none was present in this case. On the other hand in the kidneys of Monkey 6 practically all of the original epithelium of both proximal and distal convoluted tubules must have been destroyed before the last dose was given, as evidenced by the presence of numerous new and uninjured cells and but few of the original type. All of the latter cells exhibit varying degrees of necrosis. It is significant that histological evidence of injury to regenerated epithelium is lacking, in spite of repeated injections of increasing size, while the original cells display frank and widespread necrosis. In this respect our observations do not coincide with those of Suzuki, who found a lack of resistance of newly formed tubular cells following bichromate poisoning. Our results indicate that such cells are resistant to potassium bichromate in much the same manner as Hunter^{12, 13} had previously observed following the use of uranium and mercury.

Another interesting feature is the involvement of both proximal and distal convoluted tubules so strikingly apparent in Monkey 6. A careful study of the epithelial covering of these tubules reveals many cells in different stages of mitotic division, often already sur-

rounded by fully formed basophilic cells, but sometimes standing alone and stretching out to cover a partially persistent defect. The dividing nuclei belong to cells of the regenerated type, as shown by the strongly basophilic cytoplasm, a feature peculiar to renal epithelium formed after destruction of the original cells. From personal experience with uranium and mercury nephropathies we are able to say that cytoplasmic basophilia persists over a period of many months and is of greater value in distinguishing new from original cells than any other single criterion. Not infrequently the regenerated cells of bichromate and bichloride kidneys re-assume to a certain extent the normal cuboidal form, but the bluish coloration of the cytoplasm remains as a permanent marking. The importance of this feature seems rather generally to have been overlooked, although it was depicted clearly by Thorel in 1903 and again described by Oliver in 1915.

A further point of much interest is the morphology assumed by regenerated tubular epithelium. Long ago Suzuki noted the flattened character of tubular epithelium formed after uranium poisoning and advanced the opinion that it might represent an ingrowth of cells from the thin limb of Henle. Somewhat later Oliver made the same observation as to the morphology, but felt that flattening was due to hindrance by the connective tissue proliferation which developed conjointly with the epithelial changes. Oliver saw even in severe cases where the tunica propria was practically denuded a persistence of a few cells with normal nuclei but altered cytoplasm, from which he believed regeneration took place. An occasional mitotic figure was found in the slightly damaged first division of uranium kidneys, but there was even less proliferation of the cells lining the thin limb of Henle's loop. Later still Hunter¹² confirmed Oliver's observations. Quite recently MacNider¹⁴ has asserted that in dogs poisoned with uranium nitrate more than one kind of repair may be instituted to replace the loss of cells in the proximal convoluted tubules. When the initial injury was not too severe, as measured functionally by the severity of response and rapidity of recovery, the type of repair was found to be affected by the formation of cells from preëxisting tubular epithelium normal for the location of the injury. A subsequent injection of uranium caused necrosis of this normal form of regenerated cell and indicated a lack of resistance or even in-

creased susceptibility if the dogs were pregnant. A second variety of repair consisted in a replacement of convoluted tubular cells by flattened atypical forms "originating as an ingrowth from below . . . less specialized in structure and apparently in function" (see page 429). The latter form MacNider recognized as resistant to uranium and felt that its development might constitute a part of the defense mechanism of the kidney. Although MacNider does not state the exact source of the flattened cells the inference is that the flattened cells come from the descending limb of Henle's loop. We are unable to subscribe to this view, but rather agree with Oliver that flattening is probably the result of connective tissue proliferation, which we have found not only after damage of the kidney by uranium, but also to a slight extent with potassium bichromate. Further, it is not unreasonable to assume that regenerating renal tubular epithelium may possess the faculty of elongation in order to fill in defects quickly and establish continuity in much the same manner as Hartwell¹⁵ has shown takes place in the healing epidermal wounds. Still another factor tending to produce flattening is pressure exerted by casts within the lumina of affected tubules. Moreover, as we have described in one animal (Monkey 6), not only is the entire proximal convoluted tubule lined by cells of the atypical, and in part flattened, type but the distal convoluted tubule as well. If then, as is true in this instance, the ascending limb of Henle's loop has normal epithelium it would be quite impossible for an ingrowth of cells from below to take place. Certainly the new epithelium covering the distal convoluted tubules must have originated from preëxisting cells at this point, yet the morphology of the cells is identical with those in the proximal convoluted tubule.

Frequently the newgrowth of epithelium in bichromate nephropathy is excessive, the cells heaping up in mounds forming syncytial giant cells or being laid down in layers. Although the intent of such regenerative activity is good, harm may result from narrowing or occlusion of the lumen.

A point of great similarity between the renal picture of bichromate and mercuric nephropathies is the failure of necrotic cells to be swept out of the tubules promptly. Often remnants remain at the site of injury for some time after regeneration has begun and are bridged over by new cells, giving the impression of phagocytosis.

The inclusions are readily recognized by their strong eosinophilic staining, contrasting sharply with the bluish cytoplasm of the regenerated epithelium.

Potassium bichromate, like uranium nitrate and mercuric chloride, produces glomerular changes which are demonstrable with the aid of the azocarmine stain. A detailed description of these alterations will be found in another paper.¹⁶ In this communication it is sufficient to state that in acutely damaged kidneys there is a diminution in the number of glomerular epithelial and endothelial cells, hyaline droplet degeneration, and sometimes fraying and beading of the capillary basement membrane. The chronic stages are marked by an increase of epithelial cells, thickening, fraying and splitting of the basement membrane. In the small series of monkeys studied the glomerular lesions are less constant and less severe than those in rabbits receiving uranium nitrate and mercuric chloride.

SUMMARY

1. Potassium bichromate in dosages employed in the foregoing experiments with monkeys affects diffusely the proximal and distal convoluted tubules and glomeruli of the kidney. The type of injury is quite comparable to that induced by uranium nitrate and mercuric chloride.

2. The drug is locally corrosive when administered subcutaneously and the quantity absorbed varies considerably in different animals of the same species. Definitely chronic renal lesions were obtained in but one animal out of six.

3. Following repeated injections of potassium bichromate there occurs a regeneration of tubular epithelium of distinctly atypical morphology which is resistant to further injury by the nephrotoxin.

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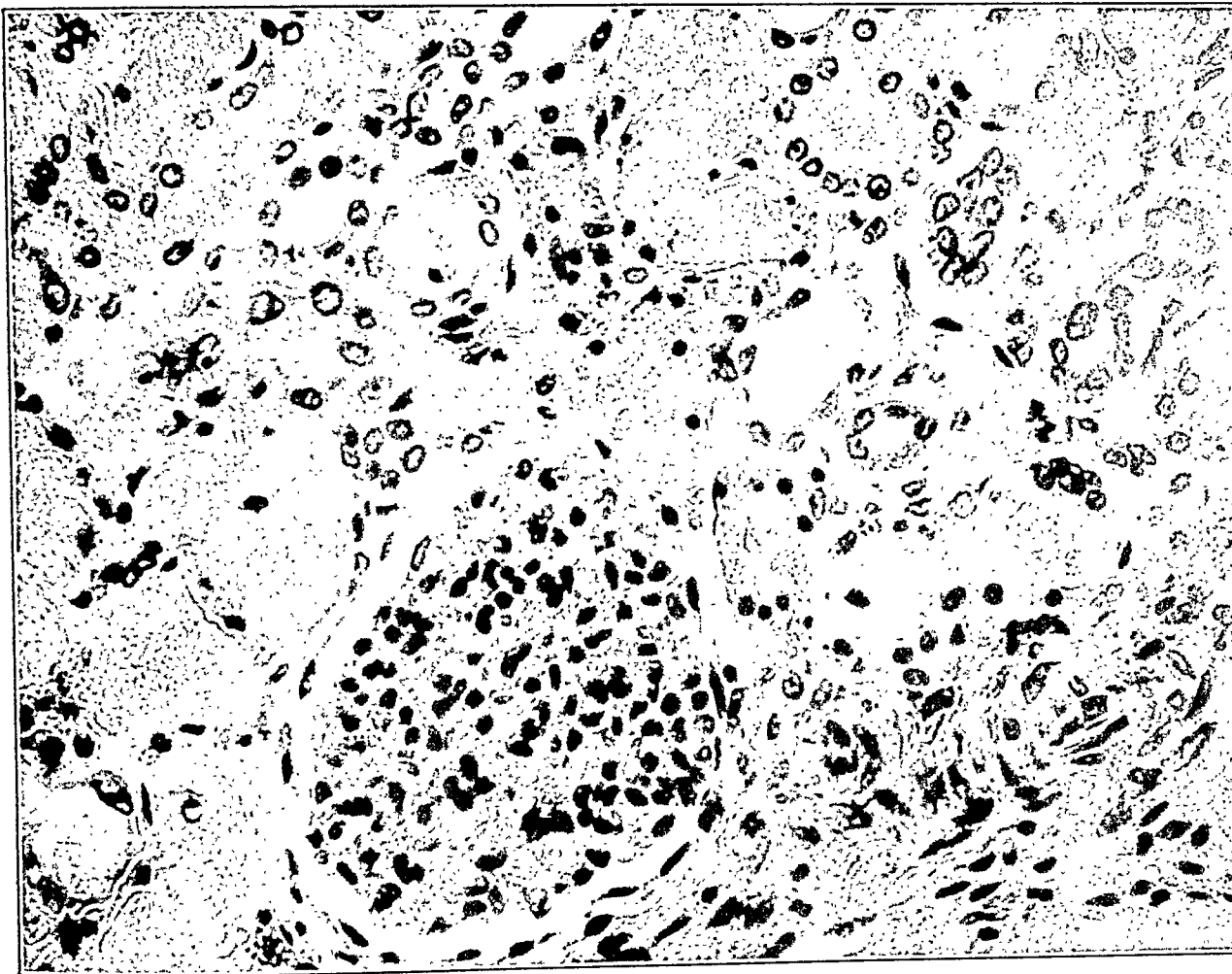
DESCRIPTION OF PLATES

PLATE 24

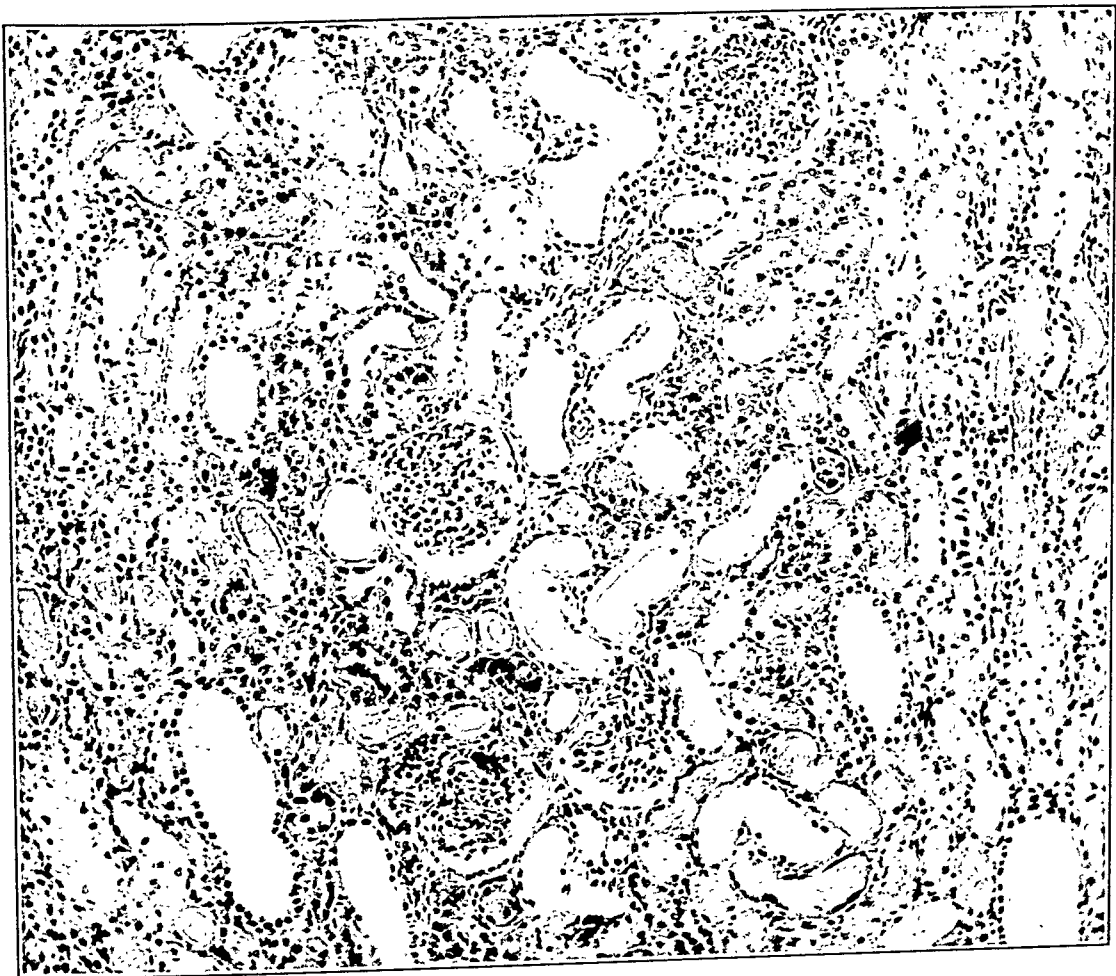
FIG. 1. Photomicrograph of a representative area from the kidney of Monkey 5.

Three segments of a convoluted tubule to the left of the glomerulus show complete epithelial necrosis, leaving only the barren membrana propria and nuclei of interstitial cells to mark the outlines of the tubules. Alongside these and near the corner is a segment of a tubule showing the hyperchromatic nucleus and scanty cytoplasm of a newly formed epithelial cell. To the right of the glomerulus are two convoluted tubules fully relined with atypical regenerated epithelium, and slightly below them is another containing a syncytial multinucleated giant cell. Three convoluted tubular segments in the upper right hand margin exhibit evidences of epithelial injury — nuclear pyknosis and karyolysis and swelling of the cytoplasm.

FIG. 2. Low power photomicrograph from the kidney of Monkey 6 showing a labyrinth with portions of a medullary ray on either side. Few, if any, proximal or distal convoluted tubules retain the original form of epithelium, but instead are lined by flattened or irregularly shaped cells. Certain of the tubules are dilated and contain hyaline casts, others are filled with granular debris. Hematoxylin and eosin stain.



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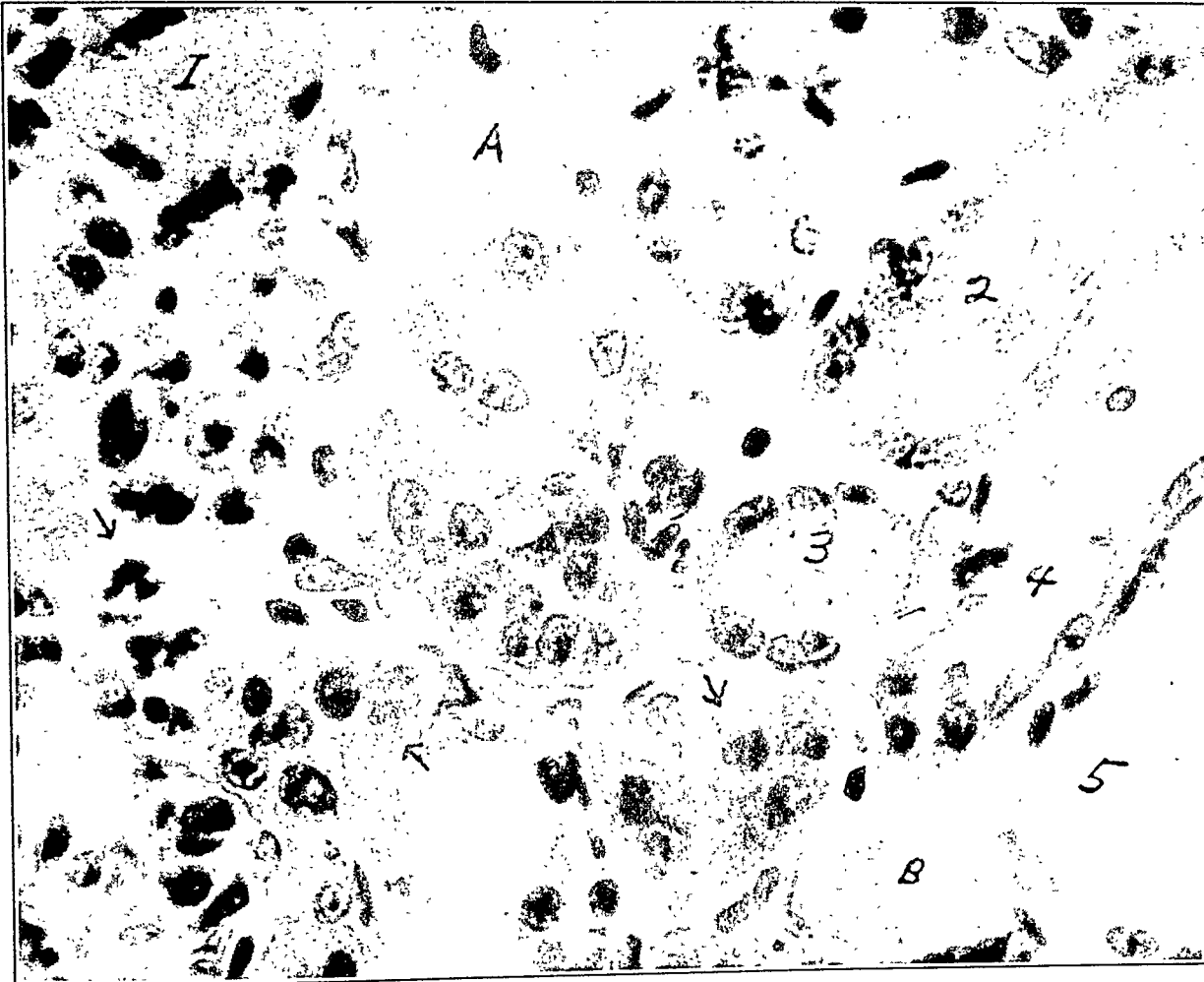


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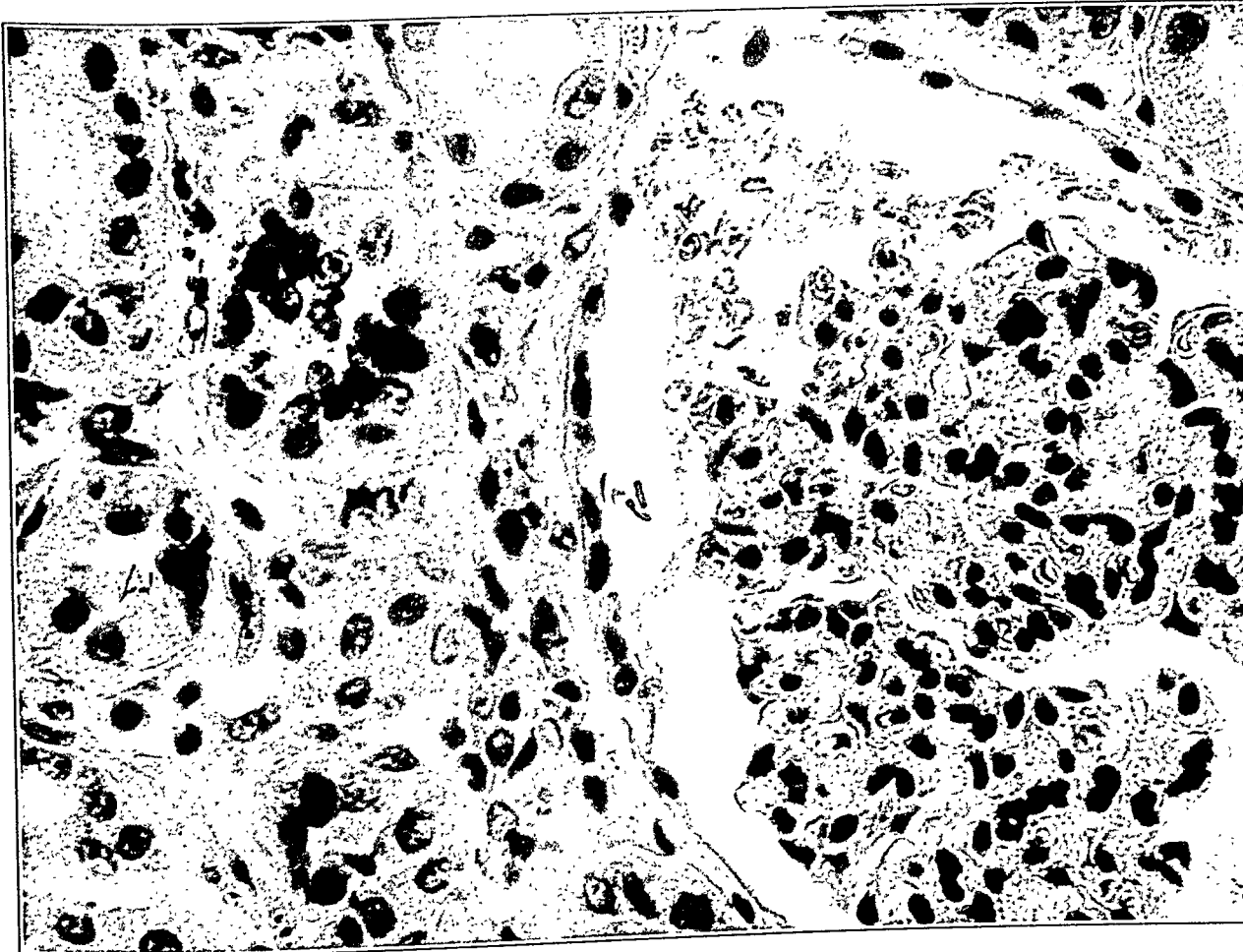
PLATE 25

FIG. 3. Monkey 6. Photomicrograph from a section stained with hematoxylin and eosin. High power. The varied morphology of regenerated convoluted tubular epithelium is well exemplified in this field. The lining epithelium of the three tubules containing mitotic nuclei (indicated by arrows) is distinctly irregular and almost occludes the lumina. In Tubules 1, 2, 3, 4 and 5 the regenerated cells are quite flat and their basophilic cytoplasm stains deeply. A and B are tubules in which complete necrosis has occurred. Note the lack of evidence of injury to regenerated epithelium.

FIG. 4. Another field from the same section as Fig. 3. Note erythrocytes and albumin in subcapsular space, pyknosis of glomerular endothelial and epithelial nuclei and slight increase of interstitial tissue external to glomerulus. On the left are three convoluted tubules almost occluded by an overgrowth of atypical regenerated epithelium. The arrows point toward a fragment of a necrotic cell over which new epithelium with dark basophilic cytoplasm is beginning to grow.



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THE MICROINCINERATION OF INTRANUCLEAR INCLUSIONS IN YELLOW FEVER *

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The method of microincineration offers a new means of approach for studies on the microchemistry of the nucleus. By burning away all the protein the hitherto insurmountable obstacle to the investigation of the mineral constituents is removed. Principal credit for the introduction of the technique is due to Professor A. Policard of Lyon. Improvements have been made by Scott ¹ who, by studying the distribution of minerals in and about the chromosomes during cell division, has greatly extended our knowledge of this phenomenon. The same author ² has found that the intranuclear inclusions caused by the submaxillary virus leave little or no ash, whereas the nucleoli of the affected cells are very rich in minerals. This has extended our ideas of the composition of the inclusions and has afforded a practical way of easily distinguishing them from nucleoli. It has also raised the question whether or not other nuclear inclusions in virus diseases behave in the same manner. The inclusions typical of yellow fever were selected for study because of previous experience with them by other methods (Cowdry and Kitchen ³).

MATERIAL AND METHODS

The material consisted of pieces of liver, taken from a monkey sacrificed in a typical attack of yellow fever, fixed in absolute alcohol containing 10 per cent of neutral formalin, and very kindly sent to

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me by Dr. S. F. Kitchen of the International Health Division of the Rockefeller Foundation. The livers of monkeys not infected with the virus of yellow fever were used as controls.

It is unnecessary to give the technique of microincineration in detail for it has already been described in this Journal by Covell and Danks⁴ and by Danks⁵ in their reports on the cytoplasmic inclusions in rabies and in fowl-pox.

In this study serial paraffin sections were made 4 microns in thickness and mounted on slides. The sections on alternate slides were treated differently. The first were mounted in the usual fashion with egg albumin and were colored by Giemsa's stain. These served as controls, showing the appearance of the intranuclear inclusions as ordinarily seen. The second were mounted and incinerated in accordance with instructions by the investigators named, but for the guidance of others who may wish to repeat my observations two points may be noted.

Since nuclear material incinerates less readily than that of the cytoplasm, it is important to make sure that the incineration is complete. The following description relates to sections on slides placed in the electric oven and held between 95-100° C for ten minutes. The temperature was then gradually increased at the rate of approximately 100° C every five minutes to between 600 and 620° C, when they were allowed to cool and were covered. The ashes were examined in the dark-field and their appearance depended to some extent upon the light used and the optical equipment employed.

The source of illumination was a large-sized Spencer microscopic lamp (No. 394), fitted with a Projection Mazda, 500 W., 115 V. General Electric bulb. The distance between the filament and the mirror of the microscope was 18 inches. A piece of white frosted glass about 1 mm. thick was inserted at the end of the microscopic lamp near the mirror in place of the so-called "daylight glass." The light was accordingly rather yellow. Both the incinerated and the control-stained sections were examined with a Zeiss binocular mon-objective microscope. For the former a Zeiss cardioid condenser was employed and for the latter a condenser of numerical aperture 1.40. The objective was a 3 mm. "X" with iris diaphragm, and paired compensating oculars X 10 were used for routine study.

OBSERVATIONS

In order that the results of microincineration may be viewed in proper perspective it is necessary to lead up to their consideration by reference to the appearance of the nuclei in the fresh unfixed condition and in stained preparations. This preliminary account will be based largely on a previous paper (Cowdry and Kitchen³). The steps in this transition from still living to fixed and stained, and to fixed and incinerated nuclei are illustrated diagrammatically in Plate 26 by three pairs of figures, 1 and 2, 3 and 4, and 5 and 6 respectively. The three in vertical column on the left represent normal nuclei viewed in these three states and those on the right, nuclei containing inclusions under corresponding conditions. It is obviously impossible to examine a nucleus in physiological saline; to fix, section, stain and re-examine the same nucleus; and finally to destain, incinerate and study it again for the third time. For the sake of clearness, however, the diagrams have been prepared as if this had been feasible, that is to say, nuclei of about the same size and showing approximately the same structure have been presented. The injured nucleus selected for this comparison represents a typical intermediate stage in the formation of inclusions, not the beginning or the end of the process. To save space on the plate, the cytoplasm has been omitted.

When the nucleus of a normal liver cell is examined in the living condition with high magnification and strong direct light, the nuclear membrane and the nucleolus can be distinguished easily, but the nucleoplasm appears to be of the same refractive index throughout its extent so that optical evidence is lacking of structural differentiation (Fig. 1). In the case of a nucleus altered by the virus of yellow fever, on the other hand, distinct nuclear inclusions can be seen in addition to the nucleolus (Fig. 2). These inclusions consist of clumps of tiny spherules of fairly uniform size. They occur usually to one side of the eccentrically placed nucleolus and separated from it and from the nuclear membrane by nucleoplasm in which no structure can be made out.

In fixed and stained preparations further details are noticeable in both normal and injured nuclei (Figs. 3 and 4). It is assumed that they are due to the accentuation by stains of the changes brought about by the fixative. In the normal (Fig. 3), the inner surface of

the nuclear membrane is no longer smooth and even, but is encrusted with basophilic material which is also found free in the nucleoplasm and in association with the nucleolus. This is represented in black. If the stain is suitably differentiated, a small amount of acidophilic material is also visible in the nucleoplasm and the nucleolus can be seen to have an acidophilic core. For contrast, these acidophilic components are depicted in gray. Of the two, the nucleolus certainly exists in approximately the same form in living cells, but the scattered acidophilic material in the nucleoplasm is probably, like the basophilic substance, a product of the coagulation of material originally distributed somewhat diffusely in it. The nuclei of cells injured by the virus of yellow fever, when stained in exactly the same way, present a very different picture (Fig. 4). Most of the basophilic chromatin is margined irregularly on the nuclear membrane and comparatively little of it remains free in the nucleoplasm. The nucleolus is more rounded and has lost a good deal of the basophilic chromatin attached to it. The scattered particles of acidophilic chromatin are difficult to distinguish in the nucleoplasm. They may have completely disappeared or may be masked by the clumps of acidophilic particles making up the nuclear inclusions. The latter are very conspicuous structures and easily identifiable, but they are not so evenly rounded and uniform in size as when viewed in the fresh state.

In the incinerated normal nucleus (Fig. 5), the ash on the nuclear membrane is clearly that remaining from basophilic chromatin. The same interpretation is justified for the larger heavy masses of ash in the nucleoplasm and for the ash attached to the nucleolus. The few, small, feebly refractile clumps of ash in the nucleoplasm may represent the remains of the scattered acidophilic chromatin. The identification of the large, dense, ovoid mass of ash with the acidophilic nucleolus is also evident. In the injured nucleus (Fig. 6), incineration gives a heavy ash on the nuclear membrane, which remains from the burning of margined basophilic chromatin (Fig. 4), which in turn is probably a coagulum of more diffusely distributed substance brought about by the fixative, as it is not visible in the living condition (Fig. 2). The nucleolus yields a very compact deposit of white ash which is similar in shape to the acidophilic core (Fig. 4). About this deposit is a lighter ash which remains from the basophilic chromatin associated with the core. In the nucleoplasm

there are a few scattered ashes as residues of depleted chromatin in this situation, but it is unsafe to hazard an opinion as to whether this chromatin was basophilic or acidophilic in nature. No definite traces are to be seen of the inclusions which were a prominent feature of the altered nucleus when observed in the living state (Fig. 2), and after fixation and staining (Fig. 4).

The description may now be extended to include an account of the liver cells reacting to the virus from the first to the latest stages, as seen after microincineration, and in alternating sections colored by Giemsa's stain. The figures on Plate 27 were drawn at the level of the table with objective "X," compensating oculars X 20, and camera lucida. They illustrate the cytoplasmic ash as well as the nuclear ash.

The cell membrane shows up in the dark-field as a glistening line of white ash. The line is, however, not of equal thickness throughout. It is made up of small clumps of ash which are unevenly spaced. Between such clumps no traces of the membrane can be made out although alternate stained sections of corresponding cells indicate that the membrane is continuous. This may mean: (1) that during life the mineral constituents are irregularly distributed with reference to the membrane, or (2) that the clumping is an expression of the coagulation that occurs when the tissue is fixed, or (3) that it takes place during the process of incineration. In later stages involving necrosis, rupture of the membrane is indicated by lines of ash pointing in different directions (Fig. 14). The cytoplasm leaves a greater residue in the central and peripheral zones of the lobule than in the intermediate zone which is most affected by the virus. Figures 7-9 illustrate cells in the peripheral zone and Figures 10-14 in the intermediate. It will be observed that for similar volumes the cytoplasm shows distinctly less mineral than the nucleus, and also that it disappears more rapidly as necrosis advances.

The nuclear membrane is likewise outlined by an interrupted ash deposit. In Figures 7-9 its contours can, however, be clearly made out. In the cells represented in Figures 10-14 the nuclei are shrunken and to some extent fragmented. Figure 10 shows a heavy accumulation of ash in or on the lower part of the membrane as it is illustrated. No remains of the upper part are to be seen. The ash is also very marked in Figure 11, in which the nuclear membrane appears to be breaking up. Further shrinkage is indicated in Figures 12 and 13,

while only traces of the membrane remain in the cell shown in Figure 14.

The nucleolus leaves a much heavier and more compact ash than any other constituent of the liver cell. In Figure 7, which illustrates but slight injury, it can be seen to be made up of a dense core, on the upper left margin of which some less compact ash can be observed. Traces of this less dense ash are also revealed in the binucleated cell represented in Figure 9. In Figures 8, 10 and 12, however, only the spherical core can be distinguished. This core evidently remains without noticeable change, while marked alterations occur in the cell membrane and in the cytoplasm, as well as in the nuclear membrane, but it usually disappears before all traces of the nuclear membrane are lost.

The nucleoplasm is of special interest because it is in it that the inclusions can be easily observed in fresh preparations and in fixed and stained ones. After incineration of nuclei only slightly injured by the virus, as judged by their plump appearance and the relatively normal cytoplasmic ash, there is no accumulation of ash detectable, by the methods used, in the intermediate zone of nucleoplasm where we have reason to believe early inclusions existed (Fig. 7). The scattered particles of ash which persist do not correspond with the inclusions seen in similarly injured cells in alternating stained sections. They tend to be rod-like and angular, vary considerably in size and are often massed near the ash of the nucleolus and of the chromatin associated with the nuclear membrane, leaving the intermediate zone of nucleoplasm clear. In all probability they are the residue of the small amount of chromatin remaining in the nucleoplasm. Figure 8 illustrates a stage, if anything, slightly more advanced. To the left of the compact ash of the nucleolar core can be made out two very faint spherical masses of ash which occupy the position usually taken by the inclusions. Though they are somewhat larger than the individual particles which make up the inclusions it cannot be asserted positively that they are not inclusions. Such appearances are, however, very rare. The conclusion is justified that in the vast majority of cases the inclusions leave no mineral residue detectable after the incineration specified, and with the illumination and optical system employed. With less complete incineration microscopically visible residue might remain, consisting partly of organic material. Figures 10-14 show much more severe

nuclear injury. In Figures 10 and 13 a little mineral is illustrated in the nucleoplasm, which probably represents the last remaining chromatin. Examination of Figures 11, 12 and 14 shows nucleoplasm which is apparently devoid of ash (except of the nucleolus, Fig. 12), although the alternating control stained sections reveal the fact that nuclei in these conditions are packed with acidophilic inclusion material. Colored illustrations of nuclei in correspondingly advanced stages leading to karyorrhexis (Fig. 14) are given by Cowdry and Kitchen³ in their Figures 22-24 and 42, inspection of which shows masses of characteristically particulate, acidophilic material partly enclosed by marginated basophilic chromatin which is represented in these incinerated nuclei by dense ash.

The mineral residue, which thus far has been referred to simply as "ash," consists of oxides of various elements. Most of it is amorphous and not crystalline, probably because some water vapor unavoidably gained access to it after incineration before it could be covered and sealed with paraffin. Those who have had experience in microincineration are of the opinion that calcium can be identified in the dark-field by its flat white appearance, as well as by the forming of gypsum; sodium by its bluish white sheen*; iron by its faint dull red color; and silica by its birefringence in polarized light because it alone is crystalline. Scott⁶ gives a critique of the evidence. Colors are difficult to identify and measure and are conditioned to some extent by the light used in making the observations. Generally speaking, the ash associated with the cell membrane of liver cells in this study and remaining from the basophilic chromatin and nucleolus was creamy white. A tinge of red was sometimes seen in that of the basophilic chromatin and especially of the nucleolus. This was not, however, by any means constant. That it may represent iron is indicated by the application of the Bensley-Macallum test which demonstrated the presence of some iron in these substances and none in the nuclear inclusions. Cowdry and Kitchen³ found that the inclusions gave a negative Feulgen reaction for thymonucleic acid, whereas the chromatin and nucleolus yielded positive ones. We have to recall in this connection that the nucleolus in the liver cells of monkeys is more accurately described by the

* Mason's paper on "transmitted structural blue" (*J. Phys. Chem.*, 1931, 35, 73-81) indicates that this color may be caused by the physical and not the chemical properties of the ash.

term amphinucleolus, for it contains both acidophilic and basophilic material. The cytoplasmic ash was often, but again not invariably, more faintly bluish white than that of other parts of the cell. Traces of bluish ash were occasionally noted also in the nucleoplasm. Doubly refractile material was not found in the nuclei, although a little was seen along the course of the sinusoids and in the periportal connective tissue.

On the quantitative side we obviously cannot be sure that *all* of the mineral substances are completely immobilized at precisely the places that they occupy in the living cells because coagulation by the fixative, though rapid, is not instantaneous. The possibility exists, therefore, that some of the mineral may have been displaced and may even have left the cells. Policard and Okkels ⁷ have calculated that in some cases the loss may be 10-14 per cent. In a recent paper Gersh ⁸ mentions this problem of fixation and makes the following statement: "The ash observed after microincineration may and probably does represent a distribution of inorganic constituents which does not obtain during life. That the distribution has been altered will be demonstrated at another time." It would have been more helpful had he presented the evidence with the criticism.

In 1924 Policard, Noël and Pillet ⁹ reported very briefly and without illustrations the effect of different diets on the ash from sections of the livers of mice. After feeding only sugars the central parts of the lobules appeared to be much richer in ash than the peripheral (periportal). The former were much more quickly incinerated than the latter. Following a strictly protein diet the distribution of ash was uniform throughout the lobule and it was even less in amount than that in the peripheral parts of the lobules of the mice given sugar. The incineration was more difficult, the nuclei in particular remaining black for a long time. Finally, on a diet made up altogether of fat, the distribution of ash was very even and the incineration was easier than after the protein diet, and more difficult than after the feeding of sugar. No interpretation of these findings was offered.

Later, in 1931, Noël, Pigeaud and Millet ¹⁰ made a study of the mineral content of the human fetal liver at different ages by incineration in bulk and by microincineration. They found by the first method that it increased markedly between the second and fourth

month, then remained fairly constant, to increase sharply again in the last two months. Apparently the distribution of the ash was not studied in detail by microincineration for no reference is made of nuclear or cytoplasmic ash, let alone parts of these structures, but distinction was made of the residues of the periportal connective tissue, the blood vessels and their contents. Red ash corresponding to iron was observed, particularly in fetuses over 4 months, but not in relation to blood vessels. All the ash was positive for calcium by the gypsum test.

The inadequacy of our data regarding the physiological significance of the ash normally present in the liver does not detract from objective descriptions of changes in amount in pathological states. In order, however, to have some base line as to the variability or uniformity in the ash of liver cells I have examined incinerated sections of the liver of a man 44 years of age who died of double pneumonia, and of a cat, 3 rabbits, 1 guinea pig and 1 rat which were apparently normal. All of them left ash which corresponded with that of *Macacus rhesus* in the following particulars: (1) The ash of similar topography to the cell membrane was creamy white. (2) The cytoplasmic ash was by contrast pale bluish white, but the ratio of white to bluish ash in it was not constant in all the species. (3) The ash in or applied to the nuclear membrane was creamy white, as was also most of the ash in the nucleoplasm as well as that of the nucleolus. (4) Occasionally a little pale bluish white ash was noted in the nucleoplasm. (5) Of all elements in the cell the nucleolus left the heaviest and most dense ash, but the nucleoli were more pronounced in some species than in others. (6) Dull red ash was of rare occurrence but was detected in some cases in both nucleus and cytoplasm. (7) No ash birefringent in polarized light was observed either in the nucleus or the cytoplasm of liver cells. (8) Per unit volume the ash of the nucleus was more marked than that of the cytoplasm.

DISCUSSION

From the foregoing description it is evident that the intranuclear inclusions which appear in the liver cells of monkeys under the influence of the virus of yellow fever generally differ both from the nucleolus and from basophilic chromatin in the absence of detectable amounts of mineral matter. This observation supplements Scott's

discovery that the intranuclear inclusions caused by the submaxillary virus in guinea pigs are likewise ash-free.²

Since inclusions which are characteristic in the sense that they resemble the type inclusions of herpes have other microchemical properties in common in virus III disease, varicella, submaxillary disease and yellow fever (Cowdry,¹¹ Cowdry and Kitchen³), it will be interesting to ascertain if this absence of detectable mineral matter also runs through the series. If so, the technique of microincineration will afford a ready means of comparing less typical inclusions with nucleoli in the same and adjacent cells. The inclusions in Borna disease, poliomyelitis (Covell¹²), and Rift Valley disease (Daubney, Hudson and Garnham¹³) belong in this category, although the evidence may be satisfactory that they represent some sort of response to the several viruses. It is not unlikely that microincineration might bring data which would settle the status of the inclusions reported by Findlay¹⁴ in mice, with the suggestion that they may be of nucleolar nature.

In view also of the microchemical similarity of the typical inclusions named, it is natural to inquire how far theories advanced to explain them are applicable to those of yellow fever.

In 1923 Goodpasture and Teague¹⁵ made the following statement: "The constancy with which the intranuclear bodies occur and the characteristic morphological and tinctorial properties which they present in experimental lesions of herpes febrilis in rabbits have convinced us that they represent, as Lipschütz claims, the presence and growth, within the nucleus, of the specific virus of the disease. It seems probable that the virus itself may be obscured by a mantle of nuclear material which gives to the inclusions their usually homogeneous appearance and acidophilic staining quality. . . ." In another place they say that "the intranuclear bodies are essentially masses of virus." Later on Goodpasture¹⁶ reported: "It seems evident, however, that the material which constitutes the 'inclusion' may partly at least be composed of coagulated nucleoplasm which may impart the acidophilic staining property of the inclusions. It is to be noted, however, that when the minute granulations are discrete enough to be recognized as such they stain faintly basophilically, whereas the precipitate from the nucleoplasm of normal cells is more acidophilic. They are to be regarded at present as elementary bodies taking part in the structure of the

herpetic inclusions." My own studies^{11, 17} on intranuclear inclusions in herpes have not brought evidence of the existence of two kinds of material in them which could be interpreted in this way.

There is no reason to believe that the yellow fever inclusions are composed of two sorts of substances. Like those in herpes they are acidophilic and uniformly so, but the color naturally depends to some extent upon the technique employed and the extent of differentiation, or the degree of extraction of the dyes. No traces could be found of minute granulations staining faintly basophilically, which could be regarded as elementary bodies. All the available data indicate that their reactions to various tests are total responses in which all of their substance is involved. Thus they completely disappear when treated in the fresh condition with dilute acetic acid (Cowdry and Kitchen³); they apparently leave no recognizable ash and the Feulgen reaction is wholly negative, also the test for masked iron, and so on. Because as far as we can tell at present the material is not a mixture of easily recognizable substances, it does not follow that it is a single chemical compound. What concerns us is that it cannot be resolved into nuclear material plus elementary bodies by standard tinctorial methods, or by those microchemical tests which have been employed. We have to consider not only the evidence but also the balance of probability. Though we cannot *see* elementary bodies or virus within the affected nuclei, we cannot deny their presence. The burden of proof is on those who make the statement that they occur therein.

The elementary bodies are supposed to be tiny microorganisms which invade the nucleus and multiply within it. Pinkerton and Hass¹⁸ have recently shown that the *Rickettsiae* of Rocky Mountain spotted fever behave in just this way in tissue cultures of the membranous exudate of the scrotal sac of an infected guinea pig. This is an important extension of Wolbach's observation that the same *Rickettsiae* are sometimes intranuclear in ticks.¹⁹ The authors describe these intranuclear *Rickettsiae* in cultures fixed in Regaud's fluid, sectioned at about 7 microns and colored by Giemsa's stain. "The organisms varied in their staining reactions from blue through the purples to red, depending upon the degree of differentiation, but never attained quite the bright red of the granules of polymorphonuclear leucocytes." They were arranged in clusters in the nucleoplasm, separated from the nuclear membrane by definite halos. "In

some cases the individual organisms of a group could not be resolved and the clusters appeared as hyaline masses." Pinkerton and Hass say that it seems unnecessary to comment on the resemblance of these clusters, "especially when imperfectly fixed and stained, to certain of the structures of unknown nature found within cells in the so-called virus diseases. . . . It therefore seems not improbable that some of the unresolved intranuclear structure now classed as inclusion bodies may be of a similar nature."

Unfortunately I have not seen the preparations of Pinkerton and Hass, but they are illustrated by an excellent colored plate. The only cell shown in which there is a resemblance between the clumped *Rickettsiae* in the nucleus and intranuclear inclusions caused by viruses is the one in the upper left-hand corner of the plate. In it the mass has the hyaline appearance to which they call attention and it is surrounded by a halo, but they state that their sections were cut 7 microns in thickness. Had they been thinner and had the comparison with typical intranuclear inclusions been more direct after fixation in the same fluid and after mounting together on the same slide, I question whether this similarity would hold. But more important are the stages in clumping of the *Rickettsiae* and in development of the inclusions. They are entirely different. The organismal nature of the *Rickettsiae* is so clear that it cannot be mistaken, whereas exhaustive examinations made by numerous investigators of the stages in development of inclusions typical of virus action have never revealed the multiplication, clumping and fusion of bodies even remotely resembling the *Rickettsiae*.

Moreover, the intranuclear clumping of *Rickettsiae* is a very exceptional occurrence and there is an element of weakness in basing thereon an interpretation of intranuclear inclusions which are certainly very widely distributed. The authors state that it "... is the first instance in which a definite microorganism has been shown to be parasitic in clusters in the nuclei of mammalian tissues." But they have apparently only found them in tissue cultures of the said tissues. Evidence that they occur in the actual tissues of animals in experimental Rocky Mountain spotted fever is conspicuous by its absence. Wolbach¹⁹ failed to find them, though he discovered them in ticks, and Nicholson,²⁰ working in my laboratory, searched for them without success. That they do become intranuclear in tissue cultures and not in the animals may be conditioned by some

alteration in the cells occasioned by their removal from the body and their growth *in vitro*.

If the inclusions caused by the yellow fever virus do contain microorganisms akin to bacteria as elementary bodies, one would expect them, in common with most bacteria, to possess a good deal of mineral matter. The observation that the intranuclear inclusions in yellow fever do not possess sufficient mineral to leave a recognizable ash, when treated by the method specified in this paper, does not support the idea that bodies of this type occur in large numbers in these inclusions. Scott's discovery² that the nuclear inclusions caused by the submaxillary virus in guinea pigs are likewise completely incinerated, leaving no ash, places them in the same category. But not until the new technique has been carefully applied to other intranuclear inclusions associated with virus action will a statement be justified as to how general is this attribute.

The fact that some incitants of disease, which were thought to be ultravisible and were looked upon as viruses, have been proved to be tiny microorganisms should not influence our point of view more than the situation warrants. Among them, the *Rickettsiae* of heart-water²¹ and psittacosis²² may be mentioned. It may be significant that despite the most painstaking search there is not a single instance in which a virus which brings about the development of an intranuclear inclusion has been demonstrated to be organismal. Yet this is only one of several reasons why those who have been concerned chiefly in the study of viruses which produce intranuclear inclusions should vigorously question the validity of the assumption that this particular group of viruses is organismal. Viruses which cause the development of cytoplasmic inclusions are on a different basis. Some of them may be true microorganisms and others not, the probability hinging, in the absence of crucial information, upon their chemical and physical properties, their resistance to agents which are lethal to known forms of life, and so on. The investigations of Goodpasture and Woodruff^{23, 24} on the cytoplasmic inclusions in fowl-pox suggest that they contain minute organisms which are the etiological agents of the disease, but fall short of being entirely convincing. It is worthy of note that these inclusions and the Negri bodies are the only ones that have been studied by the method of microincineration.^{4, 5} Both differ from intranuclear inclusions by yielding an abundant ash. This does not signify that

they are necessarily organismal; for the evidence against this interpretation of the Negri bodies is convincing and many cellular components, which are certainly not made up of invading organisms, leave an ash, but it is consistent with the view that they may be composed of bacteria-like parasites.

Since there is no direct evidence, or reason to suppose from doubtful analogy, that the inclusions typical of the yellow fever reaction do contain any organisms in the form of elementary bodies or in any other state, we may now pass to a consideration of the possibility that they consist in whole or in part of "virus," meaning thereby what the word implies, namely, a poison. Notwithstanding the observation that virus increases in amount in localities where the inclusions are developing or have formed, to contend that this topographic association in yellow fever, herpes or any other disease where it obtains, indicates that the inclusions are masses of virus is to take an altogether indefensible position. It cannot be denied that the virus may enter the nuclei because the cells have clearly been injured and their permeability altered. In the initial stages of the reaction the nuclei are sometimes a little swollen, pointing to the intake of fluid. Moreover, the virus is a substance the constituent particles of which in a watery environment are extremely small, so that it may conceivably pass through membranes relatively freely. But the penetration of virus into the nuclei and its concentration in them in the form of inclusions microscopically visible has simply not been proved. While we cannot recognize the yellow fever virus by any specific chemical test in highly potent filtrates, the possibility of doing so in the cell, itself, is remote.

SUMMARY

1. In preparations of uninjured liver cells of the monkey made by microincineration, as specified in the foregoing pages, the nuclear ash corresponds closely in position with materials seen in the fresh cells, as well as in fixed and stained preparations. The nucleolus — easily recognizable in fresh cells by its position, shape and refractive index — is found to be amphophilic in fixed and stained specimens and to yield a very heavy, sharply localized ash after incineration. Chromatin, which is not visible as such in the still living cell but can be observed after fixation and staining in the form of basophilic

substance scattered in the nucleoplasm and applied to the nuclear membrane, also leaves a mineral residue which is rather less dense.

2. Marked alterations occur in nuclei reacting to the virus of yellow fever and in which nuclear inclusions are developing. The changes in size and shape of the nuclei, in the basophilic chromatin and in the nucleolus, described by Cowdry and Kitchen in stained preparations, can be followed with almost equal precision in the incinerated specimens because parallel modifications occur in the mineral residue. But the nuclear inclusions, pathognomonic of the disease, although conspicuous features of the fresh and fixed and stained preparations, cannot be studied in incinerated specimens for they yield little or no ash. They therefore differ from the nucleoli and from basophilic chromatin in the same way that Scott observed in the case of nuclear inclusions caused by the action of the submaxillary virus in guinea pigs.

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DESCRIPTION OF PLATES

PLATE 26

Diagrams made of the nuclei of liver cells of *Macacus rhesus* at a magnification of about 6000 diameters.

FIGS. 1, 3 and 5 represent a normal nucleus of a still living cell as seen mounted in physiological salt solution at high magnification with direct illumination; a similar nucleus after fixation in absolute alcohol plus 10 per cent of neutral commercial formalin and coloration by Giemsa's stain; and again a similar nucleus incinerated following the fixation just mentioned and examined in the dark-field.

FIGS. 2, 4 and 6 show nuclei containing inclusions developed under the influence of the yellow fever virus as observed under parallel conditions, using the same techniques.

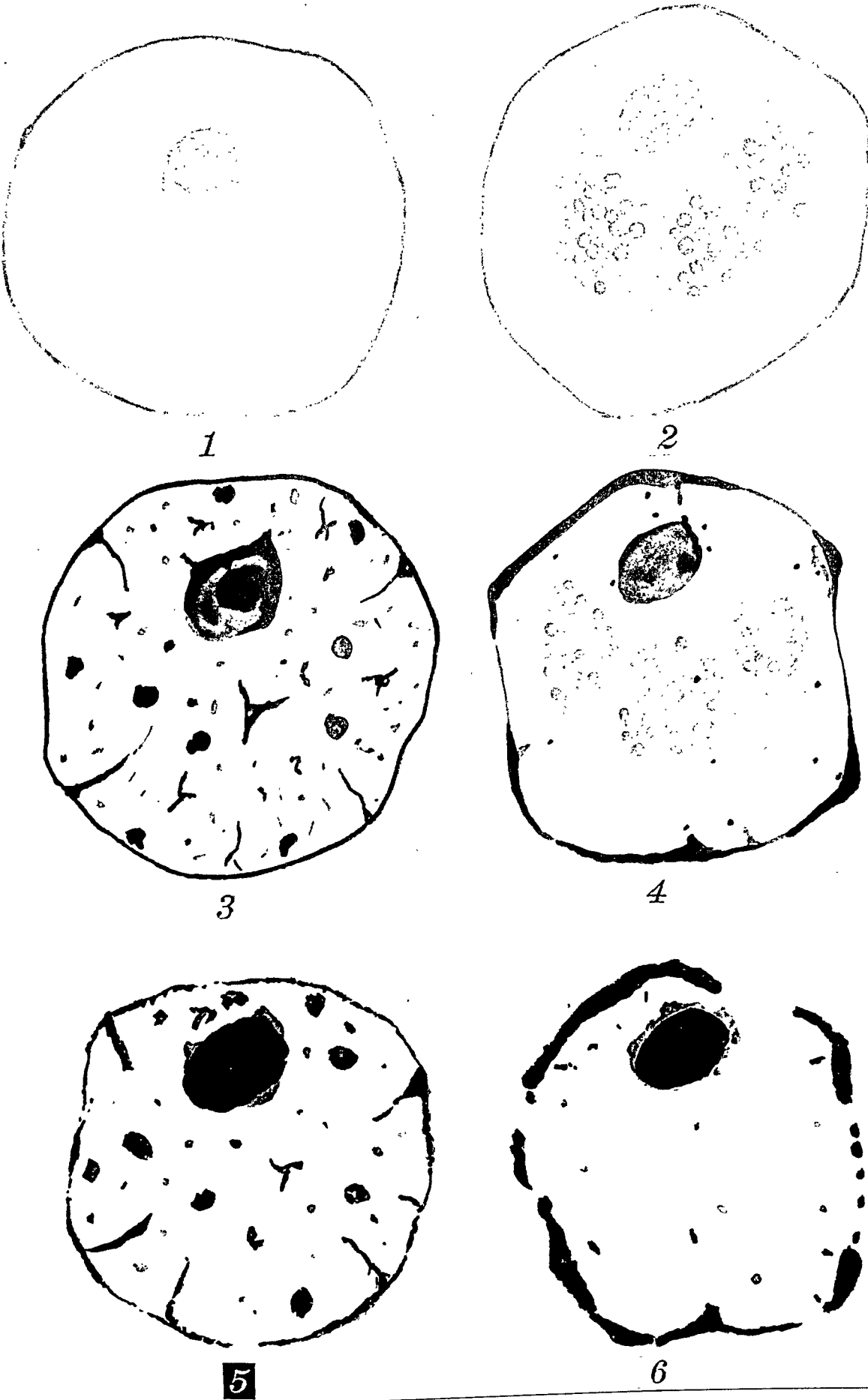
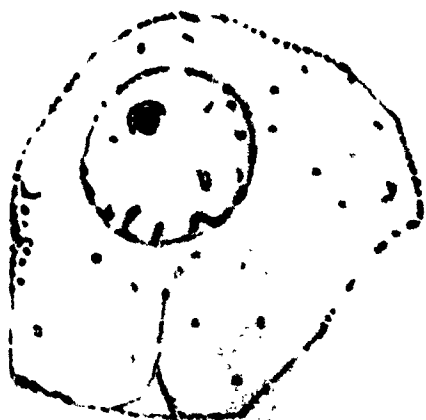


PLATE 27

Drawings of selected liver cells, viewed in the dark-field, of a *Macacus rhesus* experimentally infected with yellow fever. They were made with 3 mm. objective X, compensating ocular 20 and camera lucida at the level of the table, giving a magnification of about 1500 diameters.

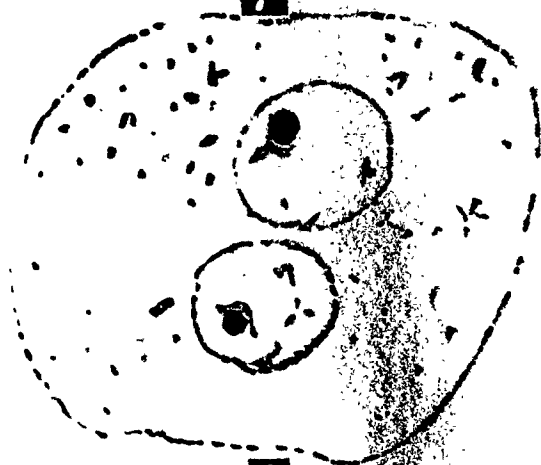
FIGS. 7-9 show the cellular ash of early stages in the reaction, and FIGS. 10-14 of later and terminal stages. The distribution of the ash clearly indicates the position occupied by the cell membrane, nuclear membrane, nucleolus and other parts of the injured cells.



7



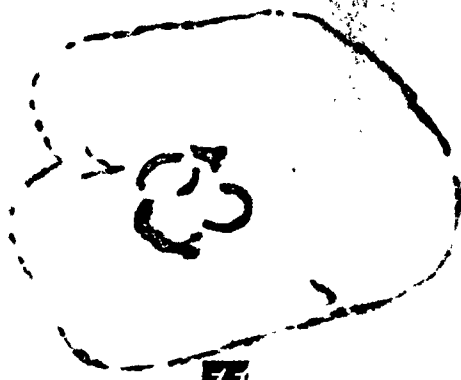
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STUDIES ON THE PATHOGENESIS OF ERYTHROLEUCOSIS *

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Erythroleucosis, a transmissible disease of the domestic fowl resembling leukemia, was discovered by Ellermann.¹ It is characterized by large numbers of basophile (lymphoid) erythroblasts in the peripheral blood, by the multiplication of these cells in the bone marrow without maturation, and by their accumulation in large numbers in the pulp of the spleen and in the capillaries of many organs such as the liver, lungs and kidneys.

Ellermann² considered this disease analogous to pernicious anemia of man. He was able to transmit it to other fowls by injections of blood, organ suspensions and cell-free filtrates of blood.

Recently most of Ellermann's experiments have been amply confirmed,^{3,4} but studies in the Henry Phipps Institute have shown that erythroleucosis has the characteristics of a neoplastic process rather than of pernicious anemia.³ It is usually accompanied by severe anemia, but it often begins with the appearance of primitive erythroblasts in the circulation at a time when signs of anemia are wanting.³ Moreover, death may occur while there is still an ample number of red cells in the circulating blood.

The investigations referred to^{4,5} have also shown that only two types of leucosis of fowls, erythroleucosis and myeloid leukemia, are caused by the same agent, a view confirmed by the studies of Engelbreth-Holm.⁶ A single animal may develop simultaneously both types of leucosis and either one may change into the other. The factors determining the types are not yet understood.

Heretofore only fully developed cases of erythroleucosis have been described. In these the changes in the bone marrow and viscera have been so advanced that the manner of their development could not be determined. The experiments reported here were undertaken to obtain information on the pathogenesis of the disease.

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PLAN OF EXPERIMENTS AND TECHNIQUE

The pathogenesis of erythroleucosis in fowls injected with cell-free filtrates of leucemic blood or organ suspensions is not necessarily the same as that caused by injections of material containing leucemic cells. With the former inoculum some blood-forming elements of the inoculated fowl must undergo neoplastic transformation, but with the second the disease may also result from the unrestricted growth of transferred cells.⁷ One group of fowls was therefore inoculated with whole blood, a second with washed blood cells, and a third with filtered plasma. These experiments were performed at a time when most of the fowls successfully inoculated developed erythroleucosis; during the earlier studies myeloid leucosis was common among fowls inoculated with this transmissible strain.

Blood smears, and in one series blood counts also, were made before the inoculation of each fowl, at 4 day intervals after inoculation, and again shortly before death (Tables I to III). The methods of blood examination employed have been described.^{3, 4} The birds were killed at regular intervals by ether; those living longer than 31 days were kept under observation for varying periods of time. Fowls were autopsied and tissue for microscopic examination was taken within 1 hour after death to determine the sequence of changes that precede the well known manifestations of leucosis. Tissues were fixed in Zenker-formol solution, embedded in paraffin and stained by hematoxylin and eosin-azur II, Heidenhain's modification of Mallory's anilin blue stain, and some by a combination of Weigert's elastic tissue stain with hematoxylin and eosin-azur II. The last method is useful in attempting to trace the endothelial lining of the capillaries of the bone marrow.

Since all but two fowls, one in the first and one in the third group, that lived longer than 15 days after inoculation developed leucosis, it may be supposed that the majority of those killed before the blood gave evidence of erythroleucosis would have developed the disease had they been permitted to live.

Two fowls in the first and two in the second group recovered from leucosis, as indicated by the study of blood smears. These, with eight other fowls surviving leucosis of varying intensities, were killed at various intervals after apparent recovery for observation of tissue changes with recovery. Baumgarten,⁸ it may be recalled, has assumed that in leucemia of man healing may be associated with osteosclerosis. It has also been stated that osteosclerosis accompanies leucosis of chickens.⁹ The frequent occurrence of bony growth

of unknown etiology obliterating the marrow cavity of fowls adds further interest to these examinations.

In the present study attention was directed to the possible origin of the basophile erythroblasts whose intravascular multiplication characterizes erythroleucosis, and to the changes accompanying erythroblastic hyperplasia of the marrow. It may be recalled that the causative agent of leucosis of fowls is, according to Ellermann and later investigators, a filterable virus. It is maintained (McGowan ⁹) nevertheless that some infections, particularly those of the respiratory tract, may extend to the marrow cavity by way of the air sacs and cause changes resembling leucosis. None of the fowls studied here had any infection of the respiratory tract.

The terms used in describing the cells of the erythrocyte series are, in the inverse order of the cellular development: erythrocyte, polychrome erythrocyte, polychrome erythroblast, basophile erythroblast. In blood smears basophile erythroblasts comprise cells varying in appearance from the typical erythroblast to those resembling primitive lymphocytes, but in sections these fine differences are lost.

In blood smears containing numerous erythroblasts, primitive cells are seen resembling lymphocytes and lacking the morphological character of erythroblasts. They were described and illustrated in a previous report ³ as "lymphoid cells." Since the available evidence points to the erythroblastic nature of these cells ³ we have included them (in Table IV) among the basophile erythroblasts.

OBSERVATIONS

In Tables I to IV only conspicuous deviations from the normal, as determined by comparison with organs of healthy fowls of corresponding ages, are mentioned. In summarizing the results of the blood examination the term anemia has been used when many polychrome erythrocytes and erythroblasts, and but few or no basophile erythroblasts, were seen in smears. The presence of numerous basophile erythroblasts is taken to indicate erythroleucosis. These designations are arbitrary. In the tables maturation of the red cell series is referred to when the term *maturation* is used, and by *stasis* is meant accumulation of basophile erythroblasts in the capillaries.

Table I summarizes observations on thirteen fowls (Group I), each of which was injected intravenously with 0.6 cc. of heparinized whole blood from a fowl with severe erythroleucosis.

Table II summarizes observations of ten fowls (Group II), each of which was injected intravenously with 0.6 cc. of washed blood cells from a fowl with severe erythroleucosis.

The cells were prepared as follows: Heparinized blood was centrifugalized at low speed, the plasma discarded and the cells resuspended in Locke's solution, spun again, and again suspended in a sufficient quantity of Locke's solution to equal the original volume. The cells were kept chilled throughout the process.

TABLE I
Changes in Organs of Fowls Inoculated with Whole Leucemic Blood

No. of fowl	Weight				Autopsy	Blood at last examination	Microscopic Examination		
	at inoculation	postmortem					Bone marrow	Liver	Spleen
		body	liver	spleen					
1656	gm. 660	gm. ..	gm. ..	gm. ..	days after inoculation 4	Negative	Normal; few erythroblasts in sinusoids with active erythrogenesis	Negative	Negative
1657	620	4	Negative	Normal; few erythroblasts in sinusoids with active erythrogenesis	Negative	Negative
1658	740	..	39.3	1.7	8	Anemia	Congested hyperplastic; normal maturation except in foci of basophile erythroblasts	Negative	Negative
1659	710	..	39.5	3.7	8	Negative	Normal except in a few capillaries containing numerous erythroblasts	Negative	Negative
1664	600	13*	Anemia	Many sinusoids filled with basophile erythroblasts; otherwise normal	Negative	Negative
1660	620	765	36.0	4.0	15	Incipient erythro-leucosis	Slight hyperplasia; basophile erythroblasts numerous in many sinusoids; normal maturation except in these; slight intercapillary fibrosis	Slight stasis	Slight stasis

1661	710	900	38.0	2.5	15	Negative	Normal except a few sinusoids which contain numerous erythroblasts	Negative	Negative
1663	730	790	110.0	11.1	22	Severe erythro-leucosis	Erythroblasts fill all sinusoids; few groups of myelocytes	Pronounced stasis	Pronounced stasis
1666	610	870	35.7	3.8	22	Incipient erythro-leucosis	Erythroblasts fill all sinusoids; no maturation	Slight stasis	Slight stasis
1662	630	1030	46.3	2.0	31	Negative	Congestion and hyperplasia of red and white cell-forming tissues; normal maturation	Negative	Negative
1665	605	930	69.5	7.8	31	Erythroleucosis	Erythroblasts fill entire capillary bed; no maturation	Stasis	Stasis
1667	550	1180	47.4	3.5	86	Negative	Congestion; marked hyperplasia of all elements; normal maturation except in several large foci which are filled with erythroblasts	Negative	Perivascular necrosis; myeloid hyperplasia
1669	620	860	40.0	3.0	183	Negative	See Table V (recovered from leucosis)	Negative	See Table V

* Died.

TABLE II
Changes in Organs of Fowls Inoculated with Washed Leucemic Cells

No. of fowl	Weight				Autopsy	Blood at last examination	Microscopic examination			
	at inoculation	postmortem					Bone marrow	Liver	Spleen	
		body	liver	spleen						
	gm.	gm.	gm.	gm.						
1843	705	750	34.0	2.2	<i>days after inoculation</i> 4	Incipient erythro-leucosis	Congested, marked erythroblastic hyperplasia; maturation retarded	Negative	Negative	Negative
1844	745	750	31.0	2.3	4	Negative	Slight hyperplasia; congested; erythroblasts numerous in foci; normal maturation except in these foci	Negative	Negative	Negative
1841	765	880	38.0	1.9	8	Negative	Slight hyperplasia; few erythroblasts in sinusoids with active erythrogenesis	Negative	Negative	Negative
1842	785	850	36.0	7.0	8	Incipient erythro-leucosis	Moderate intercapillary fibrosis; erythroblasts fill patent sinusoids; no maturation	Slight stasis	Marked stasis	Marked stasis
1846	935	1060	35.0	3.0	15	Incipient erythro-leucosis	Hyperplasia; congested; maturation except in many sinusoids where erythroblasts are increased	Negative	Slight stasis	Slight stasis
1848	800	960	36.0	7.2	15	Erythroleucosis	Diffuse erythroblastic hyperplasia; no maturation; slight intercapillary fibrosis	Slight stasis	Marked stasis	Marked stasis
1849	755	755	65.0	7.5	20*	Erythroleucosis	Diffuse erythroblastic hyperplasia; no maturation.	Stasis; tumor formation	Stasis	Stasis
1850	635	900	42.0	3.0	29*	Erythroleucosis	Diffuse erythroblastic hyperplasia; no maturation	Stasis	Stasis	Stasis
1847	750	1135	40.0	2.1	120	Negative	See Table V (recovered from leucosis)	Negative	Negative	Negative
1851	880	1600	51.7	6.0	120	Negative	See Table V (recovered from leucosis)	Negative	Negative	Negative

* Died.

TABLE III
Changes in Organs of Fowls Inoculated with Filtered Leucemic Plasma

No. of fowl	Weight				Autopsy	Blood at last examination	Microscopic Examination		
	at inoculation	postmortem					Bone marrow	Liver	Spleen
		body	liver	spleen					
1902	gm. 720	gm. 780	gm. 35.0	gm. 2.6	days after inoculation 4	Negative	Congested; basophile erythroblasts numerous in many sinusoids	Negative	Negative
1903	700	745	32.0	2.0	4	Negative	Normal; sinusoids engorged	Negative	Negative
1904	700	815	28.0	2.0	8	Negative	Normal; erythroblasts numerous in engorged sinusoids	Negative	Negative
1905	680	800	27.0	2.7	8	Negative	Normal; erythroblasts numerous in engorged sinusoids	Negative	Negative
1906	600	695	25.0	1.2	8	Negative	Erythroblasts numerous in engorged sinusoids; maturation retarded	Negative	Negative
1907	650	830	31.0	1.3	15	Negative	Slight focal erythroblastic hyperplasia; maturation reduced	Negative	Negative
1908	710	890	32.0	1.3	15	Negative	Normal	Negative	Negative
1909	720	940	33.5	1.0	15	Negative	Slight erythroblastic hyperplasia	Negative	Negative
1916	730	700	41.7	2.7	24*	Erythroleucosis	Diffuse erythroblastic hyperplasia; no maturation	Stasis	Stasis
1913	720	1070	54.6	9.6	31	Erythroleucosis	Diffuse erythroblastic hyperplasia; no maturation	Stasis	Stasis
1915	720	1200	51.0	5.1	31	Incipient erythro-leucosis	Diffuse erythroblastic hyperplasia; no maturation	Stasis	Stasis
1910	770	750	85.0	20.0	46*	Erythroleucosis	Diffuse erythroblastic hyperplasia; no maturation	Stasis	Stasis
1911	600	1830	56.2	3.2	93	Negative	Normal	Negative	Negative

* Died.

Table III summarizes the record of thirteen fowls (Group III), each of which was given an intravenous injection of 3 cc. of a Berkefeld V filtrate of the plasma of a fowl with severe myeloid leucemia.

The material used for inoculation was prepared as follows: Heparinized whole blood was chilled, and then spun at high speed (1000 R.P.M.) for 10 minutes, the plasma decanted and again spun at high speed (2000 R.P.M.) for 10 minutes, and the plasma then filtered through a coarse (V) Berkefeld filter.

Leucemic blood cells and whole blood caused leucosis sooner than filtered plasma. The character of the primary change, however, appeared to be without relation to the type of the inoculum. Accordingly typical cases from each of the three groups may be combined in describing the earlier changes.

Early Organic Changes: In healthy young fowls (3 to 4 months of age) blood formation was observed throughout the midfemoral marrow; it was particularly conspicuous in the cortical region. Erythrogenesis seemed to occur in capillaries throughout the marrow. Basophile erythroblasts were very scanty, polychrome erythroblasts and polychrome erythrocytes comprising the greater number of immature cells.

The earliest lesion in erythroleucosis consisted of an increase in the number of basophile erythroblasts in scattered sinusoidal capillaries (Fig. 1). These cells are large and spherical or polygonal in shape with relatively scanty basophilic cytoplasm distributed regularly about large vesicular hyperchromatic nuclei (Figs. 3 and 4). Changes of this nature were seen in marrows of fowls Nos. 1904 and 1905 in 8 days, and in fowl No. 1909 in 15 days, after inoculation of the cell-free agent.

Following this beginning hyperplasia of basophile erythroblasts, the other immature cells of the erythrocyte series disappeared from the involved areas. There was a single or double row of basophile erythroblasts arranged along the endothelial wall of a number of sinusoidal capillaries with only mature erythrocytes in the lumen (Figs. 1 and 3). The bone marrow of fowls Nos. 1656, 1661 and 1846 illustrate this stage of the disease. Occasionally erythrogenesis both with and without maturation was seen in a single capillary cut longitudinally.

In two fowls, Nos. 1844 and 1907, there were occasional small groups of sinusoidal capillaries filled completely with basophile erythroblasts. These foci (Fig. 2) were sharply outlined and were

surrounded on all sides by apparently normal erythrocyte-forming centers.

In fowls inoculated with cell-free filtrate of leucemic blood the process seemed to continue in the marrow until most or all of the sinusoidal capillaries were involved. More capillaries became patent, fat was correspondingly reduced, and the growing basophile erythroblasts extended into the newly opened vessels. These cells were not seen to arise from endothelium or other "fixed" cells, having no continuity with the latter. The entire capillary bed of the marrow was apparently invaded before the erythroblasts appeared in the blood stream and became concentrated in the capillaries of other organs.

Fowl No. 1906 illustrates the disease moderately advanced. In the bone marrow fat was reduced to about half and the number of open capillaries correspondingly increased. Most of the capillaries contained basophile erythroblasts, but other immature cells of the erythrocyte series were absent. The erythroblasts were not seen in blood smears taken just before death or in sections of the liver and spleen.

Fowl No. 1915 illustrates the stage in the disease when the blood and viscera were being invaded by the basophile erythroblasts. The vascular bed of the marrow in this case was completely filled by these cells.

When fowls were inoculated with whole blood or blood cells basophile erythroblasts appeared in the circulating blood before the marrow was involved to any considerable extent (Tables I and II). This may be illustrated by the following cases.

Marrow from fowl No. 1843, autopsied 4 days after inoculation, contained abundant fat except at the periphery, but there were numerous basophile erythroblasts in practically all open capillaries and these cells were also seen in blood smears. The viscera were not invaded.

The lesions in fowl No. 1846 15 days after inoculation were slightly more advanced, and the marrow contained much less fat. Erythroblasts were numerous in about half of the patent sinusoids of the marrow, but the involvement was not so extensive as in fowl No. 1906 (Fig. 3). Yet there were many basophile erythroblasts in the circulating blood and in the pulp of the spleen, but the liver was free. Fowls Nos. 1666 and 1660 are further examples of the same stage. The marrow in the former contained erythroblasts in all open capillaries. These cells were also seen in blood smears, but not in the liver and spleen. In the marrow of fowl No. 1660 there were numerous erythroblasts in many open capillaries, but they were probably not so abundant as in fowl No. 1666. Yet these cells were present in the circulating blood and there was beginning stasis in the liver and spleen.

Effects Upon Other Elements of the Bone Marrow: During the earlier stages of the erythroblastic hyperplasia the myelogenic tis-

sues of the bone marrow seemed normal. In the lymphatic tissues, however, there was in two of the early cases, fowls Nos. 1659 and 1906, accumulation of large numbers of phagocytic cells containing cellular débris.

In fully developed cases of the disease no lymphatic tissue could be seen in the marrow and granulocyte formation was slight.

Hyperplasia of the intercapillary fibrous tissue was observed in three fowls. It was pronounced in one fowl (No. 1842) and mild in two (Nos. 1660 and 1848). The capillaries throughout the marrow of fowl No. 1842 were separated by extensive proliferation of fibrous tissue. Fibrosis was most conspicuous at the periphery of the marrow where it caused pronounced reduction in the vascular bed (Fig. 5). Ossification was not observed in these areas.

Changes in the Blood and Viscera: It has been seen that in fowls inoculated with cell-free material, erythroblasts did not appear in the circulation until the bone marrow was filled with them, but that in fowls inoculated with whole blood and washed cells the erythroblasts may be present in the circulating blood and in the capillaries of the liver and pulp of the spleen before the bone marrow is involved to any considerable extent (see fowls Nos. 1843, 1846, 1666 and 1660). It was previously shown,⁷ however, that when injected into the blood stream of susceptible hosts the leucemic cells of the fowl are capable of multiplication outside the marrow.

Stasis of erythroblasts in the capillaries of certain organs, notably of the lungs, liver, spleen and kidneys, seems to begin soon after these cells appear in the circulating blood. The liver, spleen and lungs are the first to be involved. Stasis in other organs is less conspicuous.

In the livers of fowls Nos. 1660 and 1842, incipient cases, erythroblasts were seen clumped together or singly in numerous capillaries. These are examples of beginning stasis. Mitoses were frequently observed among these cells, indicating multiplication in this location.

In fully developed erythroleucosis stasis of erythroblasts apparently may cause much damage to the liver, *e.g.* fowls Nos. 1849 and 1850. In the former the accumulation of the erythroblasts in the liver capillaries amounted to small tumors, in many sites expanding the capillary walls and compressing the liver cells. This was accompanied by the formation of thrombi in the portal vein. In fowl No. 1850 parts of the liver lobules were undergoing necrosis.

Damage to the spleen was seen in two fowls (Nos. 1667 and 1669) that had recovered from erythroleucosis. This consisted of bands of hyaline material about the arteries.

The factors causing stasis of these cells in some capillaries, *e.g.* in liver, spleen and lungs, but not in others, *e.g.* in voluntary muscle, are not understood. It is noteworthy that stasis is localized in the capillaries known to be active in the removal of foreign particles introduced into the circulation.

Absence of Specific Inflammatory Changes: McGowan⁹ states that leucosis of fowls is the response of the marrow to acute infection. Recently Cash and Doan¹⁰ concluded that in pigeons infection by *Bacillus aertrycke* may simulate leukemia. This similarity consists of extreme myeloid hyperplasia associated with an increase of the circulating leucocytes. White blood counts in leukemia are, however, much higher than in *B. aertrycke* infection and the leucocytes in leucosis are of the more immature types. The relation of leucocytosis to myeloid leucosis of the fowl will be discussed in a later report.

None of the fowls studied has shown signs suggestive of acute infection.

The Origin of the Basophile Erythroblasts: As to the source of basophile erythroblasts in erythroleucosis three possibilities were considered: (a) erythroblasts of the host which the transmissible agent has entered, (b) inoculated basophile erythroblasts, and (c) the endothelial cells of the sinusoids of the marrow.

In fowls injected with whole blood or washed cells, the basophile erythroblasts rapidly multiplying in the marrow may be derived from those injected. In fowls injected with cell-free material, however, the basophile erythroblasts must arise from preëxisting cells of the marrow stimulated to growth by the filterable transmitting agent.

Since there was no definite evidence of endothelial hyperplasia in the intersinusoidal capillaries of the marrow, such as has been described in birds made anemic by starvation,¹¹ nor in the sinusoidal capillaries where hyperplasia of the basophile erythroblasts is first seen in erythroleucosis, it may be assumed that the majority of these cells are derived from similar basophile erythroblasts already present in the bone marrow capillaries. Mitoses among the erythroblasts are seen frequently, but the fixed cells have not been seen to divide.

Blood Changes Accompanying the Development of Erythroleucosis: The blood picture in advanced cases of erythroleucosis has been described and illustrated in a previous report.³ In most instances it is that of a severe anemia characterized by relatively large numbers of basophile erythroblasts (erythroleucosis of

the erythroblastic type). In some cases, however, primitive erythroblasts resembling lymphocytes³ are abundant in the circulation (50,000 to 300,000) whereas cells with the characteristic structure of erythroblasts and polychrome erythrocytes are few (erythroleucosis of the primitive cell type).

The appearance of the blood smear in each fowl studied here is stated in Tables I to III. More detailed illustrations of the early blood changes are given in Table IV.

No. 1843: Four days after inoculation there was no reduction in the number of red blood cells though the presence of immature red cells suggested incipient erythroleucosis. Note furthermore an increase in the number of thrombocytes. The bone marrow of this fowl showed in the cortical areas increase of primitive erythroblasts, a lesion suggestive of incipient erythroleucosis.

No. 1844: The second fowl of this series, killed 4 days after inoculation, had no immature cells in the circulation. The bone marrow showed a few focal accumulations of basophile erythroblasts, apparently incipient lesions of erythroleucosis.

No. 1841: The blood smear of this fowl, killed 8 days after inoculation, showed no marked abnormalities. In the bone marrow there were no lesions suggestive of leucosis.

No. 1842: Four days after inoculation erythroblasts were already numerous in the circulation of this fowl, although there was no reduction in the number of erythrocytes. Note the pronounced increase in the number of thrombocytes 4 days after inoculation followed by a sharp drop of these cells. At *postmortem* examination, changes indicative of advanced erythroleucosis were observed in the bone marrow and there was stasis of basophile erythroblasts in the liver and spleen.

No. 1848 represents a more advanced stage of the disease. Note that there were few polymorphonuclear leucocytes and the thrombocytes were practically absent from the circulation 15 days after inoculation. There was mild stasis in both the liver and spleen.

No. 1851 is given as an example of leucosis ending in recovery. The differential counts leave no doubt as to the correctness of diagnosis. Eight days after inoculation the blood smear indicated erythroleucosis, but a week later many primitive large mononuclear cells (myeloblasts according to Ellermann) appeared in the circulation, suggesting myeloid involvement. At the height of the disease the number of leucocytes was estimated to exceed 300,000, there were mitotic figures in the circulation and there was an absence of thrombocytes, which had been preceded by a transient increase. A few myelocytes seen in the blood smear of this chicken 15, 27 and 45 days after inoculation have been included among the polymorphonuclear leucocytes in Table IV. Recovery was complete, for the bone marrow showed only slight non-specific hyperplasia of erythrocytic and myelogenic elements.

Progressive hyperplasia of erythroblasts with failure to mature is naturally followed by anemia. Evidence for a primary blood destruction is wanting. The replacement of the marrow by primitive cells interferes also with the formation of granulocytes, lymphocytes and thrombocytes. Lymphatic tissues being abundant in

TABLE IV

Blood Changes in Transmissible Leucosis

No. of fowl	Time of examination	Hemo- globin (Sahli)	Red blood count		White blood count	Polychrome		Basophile erythro- blasts	Throm- bocytes	Differential Count				
			in thousands	erythrocytes		erythro- blasts	mono- cytes			poly- morpho- nuclears	mast cells	primitive mono- nuclears		
1843	Before inoculation 4 days after inoculation	47 46	2295 2355	32 28	o numerous	o 35	o 4	120 223	78 66	9 3	10 29	3 1	o 1	
1844	Before inoculation 4 days after inoculation	49 42	2415 1995	31 40	o o	o o	o o	82 105	69 52	9 17	21 28	1 3	o o	
1841	Before inoculation 4 days after inoculation 8 days after inoculation	41 45 49	2165 2470 2730	42 49 46	o 1 o	o o o	o o o	42 86 55	72 75 80	9 8 3	16 12 16	3 5 1	o o o	
1842	Before inoculation 4 days after inoculation 8 days after inoculation	42 47 41	2198 2365 1735	35 25 21	2 21 26	o 11 33	o 2 13	80 181 15	71 60 86	6 13 4	21 26 10	2 1 o	o o o	
1848	Before inoculation 4 days after inoculation 8 days after inoculation 15 days after inoculation	48 — — 29	2470 — — 1770	37 — — 25	o o 20 10	o o 9 9	o o 3 4	64 115 20 0.5	56 80 84 88	15 9 3 7	28 9 12 4	1 2 1 1	o o o o	
1851	Before inoculation 8 days after inoculation 15 days after inoculation 27 days after inoculation 42 days after inoculation 49 days after inoculation	51 — — — — —	2132 — — — — —	38 — — 300 100 —	1 13 10 many many o	o 6 18 24 12 o	o 3 3 11 2 o	72 125 3 o 9 40	85 74 70 1 35.5 76	4 7 12 1 1 3	9 16 11 1.5 1.5 8	2 3 1 o 1 9	o o 6 96.5 61 4	

NOTE: — = not done; o = negative.

other locations there is no diminution in the number of lymphocytes, but disappearance of thrombocytes and decrease of the number of granulocytes usually accompany the disease.

It is noteworthy that in all instances of erythroleucosis there was an almost complete disappearance of thrombocytes from the circulation. This decline was preceded by a transient rise with the appearance of atypical thrombocytes in the circulation (in fowls Nos. 1843 and 1842 4 days after inoculation, in Nos. 1848 and 1851 8 days after inoculation). These thrombocytes were larger than normal, more nearly round, and contained many vacuoles. They showed great individual differences in size and form (Fig. 12).

Recovery in Erythroleucosis: In an attempt to determine the successive changes that occur during apparent recovery, fowls surviving leucosis were killed at various intervals after the disappearance of immature cells from the peripheral blood.

In Table V notes are presented on the microscopic appearances of the bone marrow, liver, and spleen, together with other data on ten fowls that had recovered from leucosis of varying intensities. When hyperplasia is mentioned both red and white cell-forming tissues were involved unless otherwise indicated.

Recovery from erythroleucosis seems to be in part a reversal of the changes occurring in its development. The disappearance of leucemic cells from the peripheral blood is apparently preceded by or simultaneous with the disappearance of these cells from the capillaries of the internal organs.

Fowl No. 1667, Table I, and fowl No. 2154, Table V, illustrate the early stage in the return to normal. The course of the disease of the first fowl is similar to that in No. 1851 described in Table IV. When autopsied there were still a few immature erythrocytes in the circulating blood, but the bird was apparently on the way to recovery. Both myelogenic and erythrocytic elements of the marrow were hyperplastic, but maturation appeared normal, except in several large foci made up of sinusoids filled only with erythroblasts (Fig. 6). The capillaries of the liver were free of erythroblasts and granulocytes seemed to be forming in the pulp of the spleen. The microscopic appearances were similar in fowl No. 2154 whose blood seemed normal before death. The liver and spleen were free of lesions. The marrow was hyperplastic, but maturation appeared normal except in occasional foci of sinusoids filled with erythroblasts.

More advanced regressive changes were seen in fowls Nos. 1924 and 1994. In the former the blood was negative for 17 days before autopsy. The liver and spleen were found to be free of lesions. The bone marrow was hyperplastic, but maturation of red and white cells seemed normal. In one small area the marrow was being invaded by a solid growth of large spindle cells of regular shape with

TABLE V

Notes on Fowls Recovering from Leucosis

No. of fowl	Duration of leucosis *	Time since recovery	Microscopic Changes		
			Bone marrow	Liver	Spleen
1048	days 31	days 417	Marrow cavity partly obliterated by spongy bone; marrow hyperplastic	Negative	Negative
1669	48	118	Marrow cavity partly obliterated by bone; marrow hyperplastic	Negative	Myelogenesis in pulp, hyaline necrosis about arteries
1847	4	92	Normal erythrogenesis; very active myelogenesis	Negative	Negative
1836	42	91	Slight hyperplasia	Negative	Negative
1851	41	71	Slight hyperplasia	Negative	Negative
1923	37	47	Normal	Negative	Negative
1934	54	20	Normal	Negative	Negative
1994	24	19	Pronounced congestion and hyperplasia; tumor formation (see text)	Negative	Negative
1924	59	17	Pronounced congestion and hyperplasia; tumor formation (see text)		
2154	37	7	Pronounced congestion and hyperplasia; maturation of erythrocytes and granulocytes normal, except in a few sinusoids which contain large numbers of erythroblasts	Negative	Negative

* As indicated by blood smears. At time of death the blood smears of all these fowls seemed normal.

pale vesicular nuclei. No other tumorous growth was seen elsewhere in the body of this fowl and this small area was discovered only during microscopic examination. Its appearance (Fig. 10) suggests a neoplasm arising apparently from elements of the marrow.

The blood in fowl No. 1994 had been negative for 19 days and the liver and spleen were free of lesions. In part of the hyperplastic femoral marrow there was normal maturation of both red and white cells. In other parts there was a tumorous growth of large spindle cells with hyperchromatic pleomorphic nuclei (Fig. 9). These cells formed a coarse network enclosing numerous blood spaces. The growth apparently was expanding within the marrow and encroaching on the normal parts.

In three fowls killed 47, 71 and 91 days after inoculation, there was slight non-specific hyperplasia of both erythrocytopoietic and granulocytopoietic elements of the marrow. In fowl No. 1847 killed 92 days after the blood became negative the myelogenic tissues were hyperplastic (Figs. 7 and 8).

In two fowls, Nos. 1048 and 1668, examined 417 and 118 days after recovery, the marrow cavity of the femur was almost completely filled by growth of spongy bone in which areas of active marrow were enclosed (Fig. 11). There was scant fibrous tissue about the trabeculae. Bony changes of this nature do not seem to be an essential part of recovery from leucosis; they are not infrequently seen in older fowls. More recently we have examined five additional fowls that recovered from transmissible leucosis of from 15 to 58 days duration. The *postmortem* examination was performed from 15 to 88 days after apparent recovery and osteodystrophy was not seen in these fowls.

On the basis of these observations recovery from erythroleucosis may be visualized as follows. The multiplication of erythroblasts is reduced or ceases, as is evidenced by the lack of mitotic figures among them. Polychrome erythroblasts become numerous and are seen in division. Polychrome erythrocytes and mature erythrocytes are present in large numbers in the sinusoids so that the marrow has the appearance seen with anemia. Whether the basophile erythroblasts mature or are replaced by erythroblasts capable of maturation cannot be determined by examination of fixed material.

DISCUSSION

Blood-Forming Systems Affected: The observations previously reported ^{4, 5, 6} support the view that the causative agent of transmissible leucosis of fowls may stimulate either the erythrocytic or myelogenic tissues of the bone marrow, but not the lymphatic tissues. In the series described here there was little evidence of associated myeloblastic hyperplasia, a condition not uncommon among other fowls inoculated with this transmissible strain. Thus it is shown that the agent of transmissible leucosis is selective in its action under conditions that are, however, not yet understood.

Battaglia and Leinati¹² deny the existence of lymphatic leucosis in the fowl and state that the agent transmitting leucosis of fowls may cause a primitive cell hyperplasia resulting in erythroleucosis, myeloid leucosis, or "hemocytoblastic myelosis," but the latter type is apparently identical with lymphatic leucosis of Ellermann and other investigators.

Much of the confusion of the literature on the subject of leucosis of fowls may be traced to the large number of terms proposed to designate apparently identical cells and types of disease. Ellermann seems to have described adequately all common types of leucosis. An acceptance as far as possible of his terminology is therefore desirable. The correctness of his views on erythroleucosis and the causation of erythroleucosis and myeloid leukemia by the same filterable agent are now amply confirmed. The pathogenesis of myeloid and lymphatic leukemia of Ellermann, however, requires further investigations.

Origin of Basophile Erythroblasts: The views as to the origin of erythroblasts have recently been compiled by Michels.¹³ It appears from our material that in both secondary anemia and erythroleucosis the basophile erythroblasts increase in number. The increase is moderate in experimental anemia and is accompanied by maturation of erythroblasts; it is extensive in erythroleucosis, with little or no maturation. Neither hyperplasia nor hypertrophy of endothelium is evident in these conditions.

These studies point to the basophile erythroblast as the "stem cell" of the erythrocyte series in the adult fowl. These cells are, according to our observations, capable of rapid multiplication under the influence of the agent of transmissible leucosis as well as under the action of acetylphenylhydrazin.³ Stimulated by the former they fail to mature and erythroleucosis results; by the latter there is active maturation of erythrocytes. Whether there is also occasional formation of basophile erythroblasts from fixed cells such as endothelium remains to be proved (Stockard¹⁴).

The neoplastic character of leucemic lymphocytes of mice has been demonstrated by recent transmission experiments.¹⁵ In the fowl it has been shown by transfusion experiments with blood from fowls with myeloid leukemia⁷ that the large primitive mononuclear cells (myeloblasts, Ellermann) are capable of autonomous growth in susceptible hosts. Basophile erythroblasts apparently have the same property. Autonomous growth of the injected cells is taken to explain the rapid development of leucosis in fowls injected with cells, as compared with fowls inoculated with filtered plasma. This property also is the probable explanation of the involvement of organs before complete overgrowth of these cells in the marrow.

Effect on Thrombocyte Formation: Some of the observations re-

ported here bear on the origin of the thrombocytes. Concerning the conflicting views on the origin of the thrombocytes of the fowl see Michels,¹³ and Jordan and Speidel.¹⁶ The uncertainty of our knowledge of the origin of the thrombocyte of birds is emphasized by Forkner¹⁷ who states his failure to find these cells in the marrow. Yet our studies suggest that the thrombocytes originate in the marrow, or from elements of the marrow. A drop in the number of thrombocytes preceded by a transient rise is one of the earliest changes observed in the blood with erythroleucosis. Severe erythroleucosis is often associated with an almost complete absence of thrombocytes, preceded by the appearance of atypical forms, whether young or pathological cells. Since the bone marrow is the only organ whose function is impaired in the earliest stage of transmissible leucosis it seems probable that the formation of thrombocytes is traceable to this organ. The earliest lesions commonly affect erythropoiesis and the rapid decrease of the number of circulating thrombocytes may be due to a linkage of thrombogenesis to erythropoiesis.

Other explanations, however, may be advanced. (a) The agent may have a direct destructive action on the thrombocytes. (b) Erythroblastic hyperplasia may replace the thrombocyte-forming element. The rapidity of the disappearance of these cells from the circulation also implies that the thrombocytes have a shorter life than the red cells. (c) In erythroleucosis the thrombocytes may be prevented from entering the circulation.

The observations of Jordan and Speidel¹⁶ are noteworthy in connection with our findings. In the Salamander, *Triturus viridescens*, the spleen is the main organ for both erythropoiesis and thrombocytopoiesis, the liver for granulocytopoiesis. After splenectomy differentiation of both erythrocytes and thrombocytes takes place in the general circulation. By the presence of fine granulations, stained red by Wright's stain in the thrombocytes and their precursors, the origin of the thrombocytes, they find, can be traced to hemoblasts (hemocytoblasts) through a series of cell forms paralleling the erythrocytic series.

Recovery in Erythroleucosis: The disappearance of the enormous numbers of immature cells from the circulation and from the capillaries of the viscera and bone marrow has some analogy with regression and absorption of transplanted tumors. It is possible, however, that in recovery from leucosis the leucemic erythroblasts, unlike tumor cells, resume normal maturation. That leucemic cells may mature rapidly under favorable conditions is suggested by the tissue culture studies of Timofejewsky and Benewolenskaja¹⁸ which, however, have not as yet been confirmed.

Hyperplasia of intercapillary fibrous tissue resulting in fibrosis in some fowls and tumor formation in others was perhaps caused, directly or indirectly, by the leucemic agent. Osteodystrophia (osteitis) obstructing the marrow cavity, observed in two of fifteen fowls recovering from leucosis, was perhaps also connected with the disturbed function caused by the leucemic agent. The evidence is inconclusive, however, because of the insufficient number of control fowls (fowls not infected with the leucemic agent). Osteodystrophia was also seen among fowls not inoculated with leucemic material; on the other hand it accompanied almost all instances of severe chronic (spontaneous) anemia.

CONCLUSIONS

Under the influence of a filterable agent, the basophile erythroblasts of the sinusoidal capillaries of the marrow undergo unrestricted multiplication. The erythroblasts thus formed fail to mature. They crowd out all other elements of the marrow, secondarily invade the circulation, and accumulate in the capillaries of internal organs where they continue multiplication.

Fowls inoculated with material containing erythroleucotic cells showed growth of these cells in the blood stream and organs at a time when erythroblasts had only partly filled the capillary bed of the marrow. In fowls injected with the cell-free material the blood did not contain these immature cells until the marrow was almost completely filled by them.

With erythroleucosis thrombocytes in the blood stream are at first increased and later much diminished or absent. With disturbance of erythropoiesis formation of thrombocytes is inhibited.

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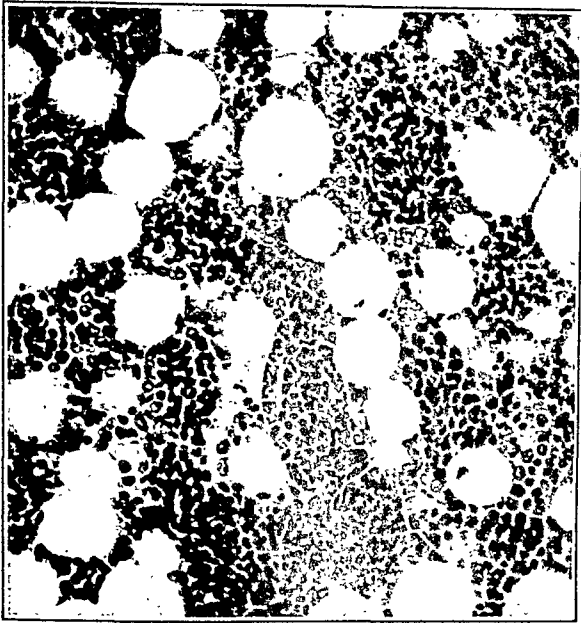
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DESCRIPTION OF PLATES

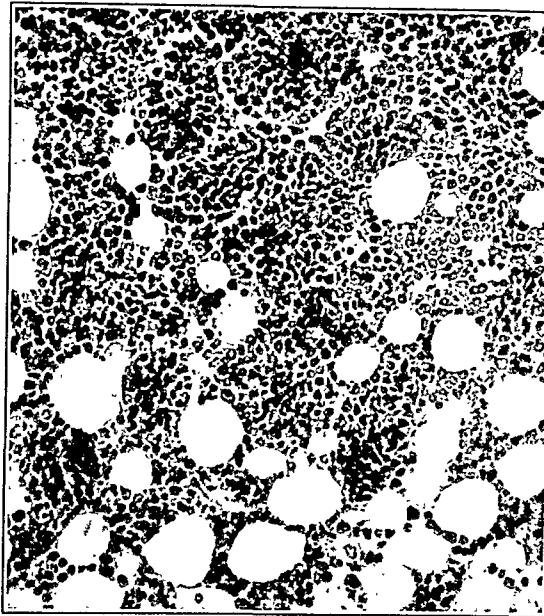
PLATE 28

Magnifications indicated are approximate.

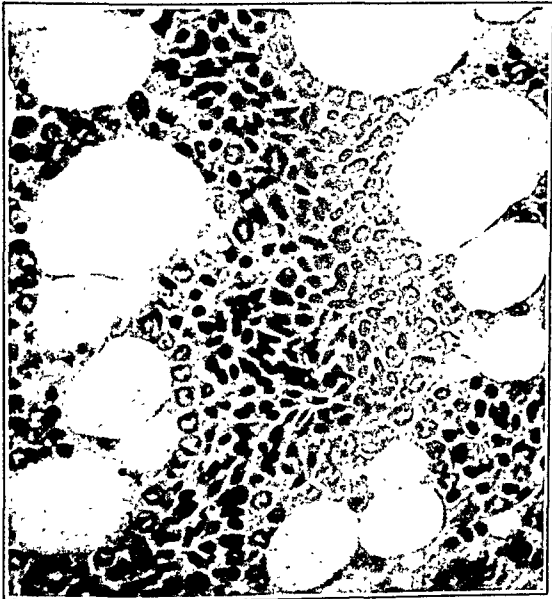
- FIG. 1. Incipient erythroleucosis: bone marrow of fowl No. 1906. Capillaries contain many basophile erythroblasts along the endothelial walls. $\times 200$.
- FIG. 2. Incipient erythroleucosis: bone marrow of fowl No. 1907. Focal erythroblastic hyperplasia without maturation. $\times 200$.
- FIG. 3. Incipient erythroleucosis: bone marrow of fowl No. 1906. A field similar to that seen in Fig. 1 shown at higher power. Note the lack of transitional forms between basophile erythroblasts and mature erythrocytes. $\times 350$.
- FIG. 4. Incipient erythroleucosis: bone marrow of fowl No. 1907. In this capillary cut longitudinally two mitoses are seen among the basophile erythroblasts, which almost entirely fill the capillary. $\times 500$.
- FIG. 5. Intercapillary fibrosis: bone marrow of fowl No. 1843. Capillaries contain many basophile erythroblasts. $\times 350$.
- FIG. 6. Latent erythroleucosis: bone marrow of fowl No. 1667. Showing the edge of a focus of basophile erythroblasts in a fowl whose blood smears indicated recovery from erythroleucosis. $\times 300$.



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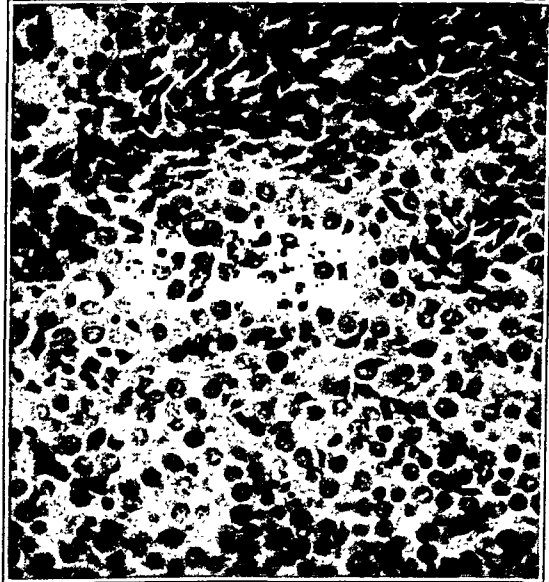
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PLATE 29

FIG. 7. Recovery from erythroleucosis, suggested in blood smears: bone marrow of fowl No. 1847, showing granulocytic hyperplasia. $\times 150$.

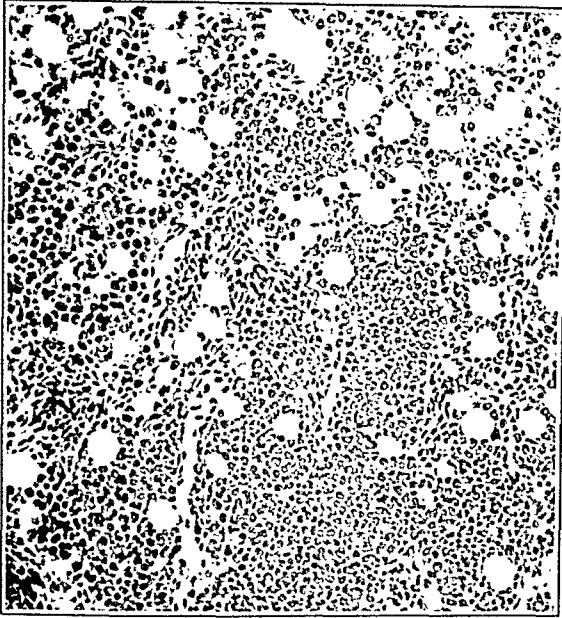
FIG. 8. Same as Fig. 7 at a higher power. $\times 300$.

FIG. 9. Sarcomatoid growth in bone marrow of fowl No. 1994, recovering from erythroleucosis. $\times 300$.

FIG. 10. Sarcomatoid growth in bone marrow of fowl No. 1924, recovering from erythroleucosis. $\times 300$.

FIG. 11. Osteodystrophia (osteitis) fibrosa: femur of fowl No. 1047, recovering from erythroleucosis. $\times 20$.

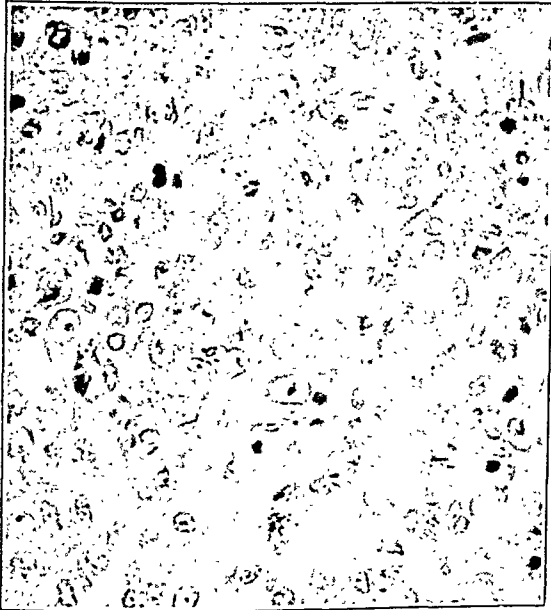
FIG. 12. Atypical thrombocytes: blood smear of fowl No. 1843, 4 days after inoculation. The central figure shows two normal thrombocytes, and the outer figure three atypical thrombocytes. $\times 900$.



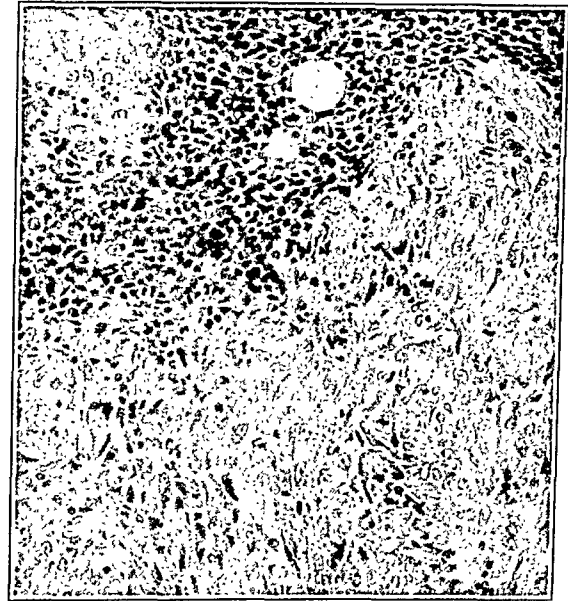
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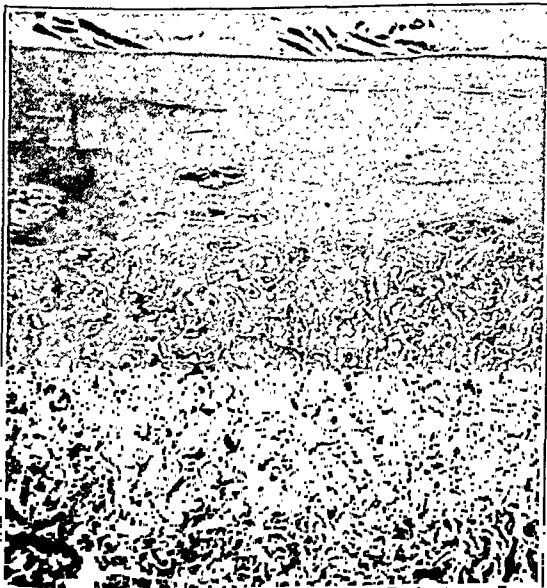
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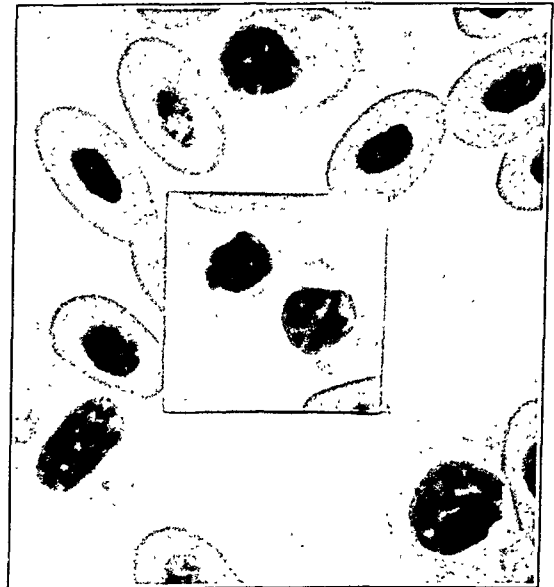
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AMYLOID DISEASE OF THE KIDNEYS *

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Minneapolis, Minn.)*

This paper is based upon a study of 65 cases of amyloid disease of the kidneys and deals chiefly with the finer structural changes in the glomeruli. Only those publications are reviewed which are concerned with structural and functional alterations in the kidneys.

It is well known that the kidneys are often free of amyloid in the presence of extensive deposits of this substance in the liver or spleen, and that in rare instances amyloid is found only in the kidneys. In Raubitschek's series¹ of 72 cases of renal amyloidosis there were 2 without amyloid in the liver or spleen. In our series of 65 cases there were 2 in which amyloid was present only in the kidneys. The circumstances that determine the site of the amyloid deposit are entirely unknown.

Forty-five cases occurred in males and 20 in females. Since the proportion of adult males to adult females is about 2 to 1 in our postmortem records, it may be concluded that sex is without influence in amyloid disease. The distribution by decades, as well as the etiological factors, is shown in Table I. Thirty-three of the 65 cases were caused by tuberculosis. In every instance the tuberculous lesions were associated with extensive chronic suppuration; there were cavities in the pulmonary tuberculous lesions, and burrowing abscesses in the instances of Pott's disease and tuberculosis of the hip.

There is general agreement that chronic suppuration is the usual cause of amyloidosis. Suppurative lesions were present in 57 of our 65 cases. In 4 instances of tertiary syphilis in our series there was chronic infection but no accumulation of pus. Amyloid has also been found with Hodgkin's disease (Bannick and Barker²), with multiple myelomas (Magnus-Levy,³ Weber⁴), and with other non-suppurative diseases. Occasionally no cause of amyloidosis is found. Fahr⁵

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reported such a case in 1918, and there were 4 in our series in which no explanation of the amyloid was found.

It may be seen in Table I that tuberculosis is a much more important cause of amyloid disease before the age of 50 years (30 of 52 cases) than it is in the later decades (3 of 13 cases).

Since the disturbance of renal function is related to the amount of amyloid deposited in the kidneys, the 65 cases have been arranged in four groups: Group A, those with only a few glomerular capil-

TABLE I

Distribution of Renal Amyloidosis According to Decades and with Respect to the Associated Disease

Associated disease		1-10 years	10-20 years	20-30 years	30-40 years	40-50 years	50-60 years	60-70 years	70-80 years	Total
Tuber- culosis	Lungs	1	3	6	10	2	..	1	23
	Spine	1	3	2	2	8
	Hip joint.....	..	2	2
	Chronic abscess	1	1	2	2	6
	Chronic osteomyelitis	1	2	1	1	5
	Empyema	3	..	1	4
	Pyonephrosis	1	1	2
	Transverse myelitis (second- ary infections)	2	2
	Tertiary syphilis	1	..	1	1	1	4
	Ulcerative enteritis	1	1
	Bronchiectasis	1	1
	Ulceration of legs	1	1
	Unresolved pneumonia.....	1	1
	Chronic arthritis	1	1
	None	1	..	2	..	1	4
Total		1	10	12	13	16	9	1	3	65

laries filled with amyloid; Group B, those with moderate amyloid deposits but without complete obstruction of any glomeruli and without tubular atrophy; Group C, those with massive deposits in the glomeruli obstructing many of the capillaries and causing slight tubular atrophy; and Group D, those with extreme glomerular obstruction and marked tubular atrophy. On anatomical grounds Group C would be expected to show a moderate and Group D a marked renal insufficiency.

With the exception of the 4 instances in which no cause of amyloidosis was found, the chief clinical symptoms were those of the major illness. The appearance of albuminuria or edema first directed

attention to the kidneys. In a number of instances of tuberculosis of several years duration there was no edema or albuminuria until a few months before death.

Albuminuria: Albumin was found in all but 4 instances in which the urine was examined. The records shown in Tables II-V are those of the last urine examination. Albumin was frequently absent until a few months before death. In 2 of the cases without albumin only small amounts of amyloid were present (Group A). In the single instance in Group B (31-1208) without albuminuria, the last examination was made five months before death, and it is probable that albumin was present later on. In the case in Group D without albumin (32-52), the record is that of a single examination made one month before death. It may be concluded that the absence of albumin in the urine is almost conclusive evidence that very little or no amyloid is present in the kidneys at that time.

The presence of albuminuria, however, in a suspected case is not sufficient evidence to warrant a diagnosis of renal amyloidosis. In 20 cases of chronic suppurative diseases in which no amyloid was found in the kidneys postmortem, the urine was free from albumin in 10 instances, contained a trace in 6, and a small amount in 4. All of these 20 cases were of the type in which amyloid commonly develops, and it was found in the liver and spleen in most of them. It is well established that a chronic toxemia may injure the glomerular capillaries and cause albuminuria without the accumulation of amyloid, but our records indicate that a heavy albuminuria in a suspected case is strongly suggestive of amyloid involvement of the kidneys. A study of Groups A, B, C and D shows, however, that there is no close correlation between the degree of albuminuria and the extent of the amyloid infiltration of the kidneys.

The albumin in the urine escapes from the blood through injured glomerular capillaries. The evidence that it escapes through the glomeruli and not through tubules has been fully discussed by Ekehorn.⁶ It will be explained later that there is evidence of injury of the glomerular capillaries in nearly all amyloid kidneys.

The Urinary Proteins: There are comparatively few studies available on the composition of the urinary proteins in amyloid disease. Geill⁷ found very low values for the albumin fraction, 35 to 60 per cent in amyloid nephrosis, whereas in chronic nephritis and lipoid nephrosis it is usually about 90 per cent. In acute nephritis he found

that the albumin fraction was at first often lower, 65 to 70 per cent, but later it increased. In amyloid nephrosis he sometimes found that globulin exceeded albumin in the urine.

Hiller, McIntosh and Van Slyke⁸ found the albumin-globulin ratio of the urine proteins usually above 10 in nephrosis, between 5 and 10 in acute nephritis, and usually below 5 in chronic nephritis with retention of urea. In 1 case of amyloid nephrosis the albumin-globulin ratio was very low, 1.5.

Lemierre and his associates⁹ studied the urinary proteins in a case of amyloid nephrosis with retention of nitrogen. Three examinations of the urine showed: albumin 6.50, globulin 1.35; albumin 6.63, globulin 1.65; albumin 15.40, globulin 9.40, grams per liter of urine.

The Serum Proteins: A few observations are available on the serum proteins in renal amyloidosis. Linder, Maxwell and Green¹⁰ found the serum proteins 3.8 to 4.7 gm. in a boy with marked retention of urea. Apparently some of the observations were made when the patient was free of edema. Bannick and Barker² studied the serum proteins in a patient with moderate renal insufficiency but without edema. The total protein was 3.8 gm. of which 27 per cent was albumin. Lemierre and his colleagues⁹ made three observations on the serum proteins on a patient who had renal insufficiency, but was not edematous: albumin 3.26, globulin 3.22; albumin 3.27, globulin 3.19; albumin 2.45, globulin 2.29. These limited observations indicate that the serum proteins are below normal in renal amyloidosis and not necessarily dependent upon edema. The heavy loss of protein in the urine would lead us to expect a depletion of the serum proteins.

Edema: Edema is a variable feature in amyloid renal disease. It was present at some time during the course of the disease in 32 of our 65 cases. It is apparently not causally related to the amyloid deposit since it is as frequent with minimum as with maximum amyloid accumulations (see Tables II-V).

Fahr¹¹ has also noted that there is no connection between the severity of the glomerular lesions and the edema. In a group with relatively slight renal changes edema was present in 4 and absent in 5; in those with severe changes it was present in 12 and absent in 4; in those with amyloid contracted kidneys it was present in 3 and absent in 2.

In 30 of our cases of chronic suppurative diseases, the majority of which had amyloid in the liver and spleen but none of which had amyloid in the kidneys, edema was absent in 12, slight in 12, moderate in 3, and severe in 3. Edema is obviously not due to the amyloid deposit, although it may be related to the glomerular injury which accompanies amyloidosis. As in other forms of renal disease edema varies in intensity from time to time. Cardiac decompensation was a factor in causing edema in a few of our cases.

Hematuria: A mild hematuria is occasionally found in amyloid disease (Bannick and Barker) but apparently it is seldom as pronounced as in acute glomerulonephritis. The erythrocytes escape chiefly from injured capillaries which do not contain amyloid.

Hypertension: The great majority of investigators find a low blood pressure in amyloid disease of the kidneys. Fahr⁵ reported 6 cases with little or no nitrogen retention in which the blood pressure was low. McElroy¹² found the blood pressure 105/70 and the urea nitrogen 9.5 mg. in his patient, a woman 33 years of age. Lemierre and his coworkers⁹ reported a constantly low blood pressure in a woman 32 years of age who died of uremia. Bannick and Barker,² in a case of Hodgkin's disease with amyloidosis in a male 38 years of age, found the blood pressure 100/70 to 115/75. The blood urea was 58 mg. and the phenolsulphonephthalein output 15 per cent in one hour. The patient of Linder, Maxwell and Green,¹⁰ a boy 13 years of age, had a blood pressure of 112/72. The blood urea reached 199 mg. Zadek¹³ reported 3 cases with uremia: a woman aged 52 years, blood pressure 130/78, blood urea 148 mg.; a male aged 63 years, blood pressure 120/60, blood urea 138 mg.; and a male aged 58 years, blood pressure 100/60, blood urea 159 mg. Rosenberg¹⁴ states that the amyloid kidney is characterized by the absence of hypertension and cardiac hypertrophy.

However, there are a few reports of hypertension in amyloid renal disease. Fahr¹⁵ mentioned 4 cases with slight hypertension. Danisch¹⁶ reported a blood pressure of 173/101 in a male 65 years of age. The non-protein nitrogen was 141.3 mg. Noble and Major,¹⁷ in a report of 3 cases with uremia, found 1 with a definite elevation of blood pressure. These 3 cases are included in Table V.

Referring to Table II it is seen that there are 2 instances of moderate hypertension in Group A. One of these shows a definite arteriosclerosis and is therefore presumably a case of primary hyperten-

TABLE II

Group A. Cases in Which Only a Few Glomerular Capillaries are Filled with Amyloid

Autopsy No.	Sex	Age	Disease	Albuminuria	Edema	Blood pressure	Heart	Kidneys	Amyloid in glomeruli	Amyloid in arterioles	Amyloid in medulla	Casts	Urea nitrogen, mg. per 100 cc.	Phenolsulphonethylate, % 2 hrs.	Comments
12-15	M	yrs.	Tuberculosis of lungs	1	—	0	200	gm.	1	1	—	1	—	—	
15-261	F	30	Tuberculosis of spine	—	—	0	151	240	1	1	—	—	—	—	
15-282	M	24	Tuberculosis of lungs	1	1	0	142	220	1	—	—	—	—	—	
15-283	M	36	Tuberculosis of lungs	1	—	0	235	353 normal	1	—	2	1	—	—	
21-494	M	13	Tuberculosis of spine	2	—	0	small	normal small	1	—	—	—	—	—	
22-74	F	58	Ulcerative enteritis	2	—	0	275	200	1	1	1	—	—	—	
23-29	M	36	Chronic abscess	0	—	0	240	370	1	1	1	—	—	—	
23-255	F	40	Tertiary syphilis	3	3	152/94	270	590	1	1	0	2	—	25	arteriosclerosis
25-508	M	36	(hypertension)												
26-420	M	75	Chronic osteomyelitis	3	—	0	300	625	1	—	—	—	—	—	
26-652	F	28	Tuberculosis of lungs	1	1	0	340	505	1	—	—	—	—	—	
27-610	F	32	Tuberculosis of lungs	1	2	110/64	200	325	1	1	1	—	23.3	—	
28-717	M	15	Tuberculosis of spine	4	1	80/50	140	300	1	1	2	—	—	—	
			Transverse myelitis	3	—	0	normal	abscesses	1	—	—	—	—	—	
			(secondary infection)												
29-1106	F	41	Tuberculosis of lungs	2	1	118/78	160	215	1	1	—	—	—	—	
29-1642	M	43	Tuberculosis of lungs	4	—	82/40	220	360	1	—	—	—	—	—	
30-65	M	16	Tuberculosis of spine	1	1	0	150	280	1	1	—	—	—	—	
31-405	M	40	Perinephritic abscess	2	2	145/90	500	255	1	1	2	1	70	—	only one kidney
31-820	M	43	Tuberculosis of lungs	0	1	128/60	342	407	—	—	1	—	—	—	
31-1559	M	39	Tuberculosis of lungs	—	1	120/80	215	350	1	1	1	1	—	—	
32-426	M	33	Perinephritic abscess	2	3	126/72	115	250	1	—	—	1	10	—	

The numerals 1, 2, 3 and 4 under the several headings in this and the following tables indicate roughly the degree of the process. 0 indicates that no observation was made. — indicates absence of the condition indicated.

TABLE III

Group B. Cases with Moderate Amyloid Deposits without Complete Obstruction of Glomeruli and Showing No Tubular Atrophy

Autopsy No.	Sex	Age	Disease	Albuminuria	Edema	Blood pressure	Heart	Kidneys	Amyloid in glomeruli	Amyloid in arterioles	Amyloid in medulla	Casts	Urea nitrogen, mg. per 100 cc.	Phenolsulphonethalein, % 2 hrs.	Comments
20-367	M	yrs. 25?	Empyema	3	—	0	gm. 170	gm. 355	2	1	2	1	norm.	50	
21-52	F	26	Chronic osteomyelitis	3	3	120/82	340	680	2	2	—	2			
21-523	F	16	Tuberculosis of spine	2	—	0	very small	large	2	2	—	1			
24-512	M	28	Tuberculosis of lungs	3	—	0	200	560	2	2	1	1			
25-1045	M	23	Tuberculosis of spine	1	—	0	normal	normal	2	2	3	2			
26-134	F	15	Tuberculosis of hip	2	—	0	small	normal	2	1	1	—			
29-652	M	78	None	1	—	150/94	350	170	2	2	1	1			
30-214	F	22	Chronic abscess	3	3	0	190	300	2	—	—	1			
30-1203	M	66	Tertiary syphilis	2	—	140/90	400	215	2	2	—	1			
30-1295	F	42	Chronic abscess	2	—	100/96	300	465	2	1	0	—			
30-1305	F	29	Empyema	0	3	120/88	not examined	294	2	1	1	1	norm.	norm.	arteriosclerosis
30-1752	M	48	Tuberculosis of lungs	3	—	86/60	218	260	2	2	3	3	29.6		
31-291	M	47	Chronic osteomyelitis	3	1	85/60	310	370	2	4	0	4	118		
31-1208	M	21	Unresolved pneumonia	—	—	116/72	300	360	2	—	0	—	17.7		
31-1356	F	35	Tuberculosis of lungs	4	—	95/68	340	370	2	2	1	1			

TABLE IV

Group C. Cases Showing Massive Deposits of Amyloid in Glomeruli with Obstruction of Capillaries and Showing Slight Tubular Atrophy

Autopsy No.	Sex	Age	Disease	Albuminuria	Edema	Blood pressure	Heart	Kidneys	Amyloid in glomeruli	Amyloid in arterioles	Amyloid in medulla	Casts	Urea nitrogen, mg. per 100 cc.	Phenolsulphonephthalein, % 2 hrs.	Comments
13-16	F	yrs. 31	Tuberculosis of lungs	0	—	0	g.m. not ex- aminated	g.m. small	3	3	3	—			
15-41	M	34	Tuberculosis of lungs	0	—	0	293	437	3	3	—	3			
20-451	M	12	Tuberculosis of lungs	3	—	0	145	325	3	1	1	1			
24-572	F	14	Chronic abscess	3	—	0	normal	large	3	1	2	3			
27-806	F	25	Empyema	3	2	0	223	445	3	1	0	2		15% (1 hr.)	
28-145	M	48	Tuberculosis of lungs	0	—	0	233	505	3	1	3	1			
28-322	M	51	Pyonephrosis	3	1	110/60	350	700	3	2	—	1			
28-545	M	18	Transverse myelitis	4	1	0	normal	very large	3	2	3	2			
29-1804	F	50	None ?	1	2	170/120	435	215	3	3	—	—	52.5		
30-533	M	23	Tuberculosis of spine	2	1	110/70	300	800	3	1	1	4			
30-982	M	46	Pyonephrosis	4	1	98/48	not ex- aminated	500	3	1	0	3	121	0	
30-1389	M	33	Tuberculosis of lungs	4	1	105/72	315	435	3	2	0	1	178		
30-1572	M	49	Tuberculosis of lungs	3	—	136/104	505	652	3	—	0	1	65		
31-1754	M	46	Empyema	3	1	238/142	375	325	3	3	1	3			
31-1877	M	43	Tuberculosis of lungs	2	—	90/58	300	380	3	3	—	4			
31-1934	F	47	Chronic arthritis	3	4	120/70	200	220	3	2	1	2	9.4	22	congo red, 56 % retention endocarditis
31-2015	M	6	Tuberculosis of spine	2	4	0	72	185	3	—	2	—			
32-362	M	43	Tuberculosis of lungs	4	3	110/90	265	400	3	1	1	—			

TABLE V

Group D. Cases Showing Extreme Glomerular Obstruction with Amyloid and Marked Tubular Atrophy

Autopsy No.	Sex	Age	Disease	Albuminuria	Edema	Blood pressure	Heart	Kidneys	Amyloid in glomeruli	Amyloid in arterioles	Amyloid in medulla	Casts	Urea nitrogen, mg. per 100 cc.	Phenolsulphonephthalein, % 2 hrs.	Comments
16-45	M	61 yrs.	Tuberculosis of lungs	2	2	0	gm. 550	gm. very small	4	4	2	2			
20-84	F	59	Tertiary syphilis	0	1	0	300	520	4	1	2	4			
21-457	M	59	Tuberculosis of lungs	3	1	0	275	370	4	4	0	1			
25-204	M	19	Tuberculosis of hip	1	3	0	normal	large	4	2	1	1			
28-64	F	35	None	3	1	190/110	355	260	4	3	0	1	147		
28-217	M	58	Bronchiectasis	2	1	0	435	240	4	1	2	4			
28-1214	M	15	Chronic osteomyelitis	3	1	0	normal	large	4	2	2	2			
28-1616	M	55	None	4	1	104/50	480	280	4	4	4	1	134	0	
28-1629	M	27	Chronic osteomyelitis	4	1	110/?	220	575	4	3	1	4	68	0	
29-1425	M	54	Ulceration of legs	4	1	120/60	580	440	4	4	2	4	134.6		
32-52	M	43	Tuberculosis of lungs	1	1	0	375	370	4	2	3	1			
32-175	M	75	Tertiary syphilis	4	4	110/64	not examined	240	4	2	1	1	N.P.N.		
													58.8		

sion. In the other case (31-405) no cause for the cardiac hypertrophy was found. It cannot be attributed to the amyloid deposit since this was minimal in amount. In Group B 1 instance of moderate hypertension was found which was presumably due to arteriolosclerosis and not to amyloid. In Groups C and D, that is, those with severe changes in the glomeruli, there are 3 cases of hypertension among 15 in which the blood pressure was recorded. In all 3 of these there are massive deposits of amyloid in the arterioles which make it impossible to decide whether there are any hyaline changes characteristic of primary hypertension. A summary of the clinical histories of these 3 cases is as follows:

29-1804 (*Table IV, Group C*): A woman 50 years of age was admitted to the hospital Nov. 19, 1929, complaining of dyspnea and moderate generalized edema. Dyspnea with a constant non-productive cough had been present for about one year, and edema about two months. There was slight cyanosis of the face, a slight bilateral exophthalmos, and a bilateral lid lag. The thyroid was not palpable. A few moist râles were heard in both lung bases. The heart was enlarged to the left, and a systolic murmur was heard at the apex. The abdomen was distended and a fluid wave was elicited. The blood pressure was 170/120. Urinalysis on many different examinations showed a trace of albumin, hyaline casts, and sometimes erythrocytes. Hemoglobin was 77 per cent; erythrocytes 4,350,000, leukocytes 6100, blood sugar 0.07 per cent; creatinin 1.8 mg.; urea nitrogen 52.5 mg.; blood Wassermann ++++. Death occurred Dec. 11, 1929. No anatomical evidence of syphilis was found at postmortem, but the positive Wassermann reaction suggests syphilis as a possible factor in causing the amyloid disease. There was no other assignable cause for the amyloidosis. There was no amyloid in the liver or spleen.

31-1754 (*Table IV, Group C*): A male 46 years of age developed empyema following pneumonia in 1919. Right thoracoplasty was performed. Four years later a sinus developed which has persisted, having been treated by irrigations. His last illness began Oct. 11, 1931, with deep-seated pain in the right chest. On October 21 he became semicomatose. On October 22 he was admitted to the hospital in coma. He had one convulsion. His muscles were spastic and the reflexes were hyperactive. The temperature was 105° F; leukocyte count 26,000; albuminuria +++; blood pressure 238/142. Death occurred Oct. 24, 1931. No determination of blood urea was made. The changes found in the kidneys support the clinical diagnosis of uremia. There was a residual empyema in the right chest.

28-64 (*Table V, Group D*): The patient, a woman 35 years of age, was admitted to the hospital Dec. 13, 1927, complaining of impairment of vision, pain in the chest, dyspnea and frontal headache. These symptoms had begun about two weeks previously. The heart was enlarged to the left and there was a blowing systolic murmur over the apex transmitted to the axilla. The blood pressure was 190/110. A few coarse râles were heard posteriorly over the bases of the lungs. The liver was definitely palpable below the right costal margin. There was slight edema of the legs. The blood Wassermann reaction was negative. The urine showed albumin from + to ++++. December 19, creatinin 3 mg.,

urea nitrogen 116 mg. December 31, creatinin 3 mg., urea nitrogen 147 mg. She had a slight fever during the last three days of life. Ophthalmoscopic examination revealed an albuminuric retinitis. Death occurred Jan. 10, 1928. The clinical diagnosis was chronic glomerulonephritis, but the postmortem revealed advanced amyloid disease of the kidneys. No cause of amyloidosis was found. The clinical picture seems indistinguishable from chronic glomerulonephritis.

It may be concluded that there are occasional instances of renal amyloidosis in which a definite hypertension develops that is presumably due to amyloid which causes narrowing of the glomerular capillaries and the arterioles of the kidneys. It is known that increased resistance in the renal circulation may cause hypertension.

Renal Insufficiency in Amyloid Disease: It is well known that amyloid disease may terminate in uremia. Cases of this type have been reported by Danisch (2 cases), Linder, Maxwell and Green (1), Noble and Major (3), Lemierre and associates (1), and Zadek (3).

In our series functional tests were made on only 20 of the 65 patients. The patient in Group A (Table II, 31-405) with a urea nitrogen of 70 mg. had only one kidney and it was surrounded by an abscess. The case in Group B (Table III, 31-291) with a urea nitrogen of 118 mg. showed only a moderate amyloid deposit, but the tubules were nearly all blocked by casts. All the cases in Groups C and D that were studied show more or less evidence of impaired kidney function. A histological study of the kidneys of these two groups reveals extensive obliteration of glomerular capillaries, which is convincing evidence of a marked impairment of kidney function. All except 3 of these patients were suffering from a well defined chronic infectious process, yet renal insufficiency must have played an important rôle in causing death.

Size of the Kidneys: In amyloid disease the kidneys are usually larger than normal, but they may be of normal size or contracted. In 41 of 72 cases Raubitschek found "large white kidneys." The average weight of both kidneys in our series was as follows: Group A, 349 gm.; Group B, 366 gm.; Group C, 452 gm.; and Group D, 366 gm. The enlargement is due in part to cloudy swelling resulting from the infection, since about half the kidneys in Groups A and B, with a relatively small amount of amyloid, are above normal size. The largest kidneys are found in Group C, in which there is the greatest amount of amyloid, but even in these swelling of the epithelial cells and dilatation of the tubules is chiefly responsible for the

increased size. In Group D with uremia there are some small kidneys, but some are large and we do not find the extremely contracted kidneys that are often seen in long-standing glomerulonephritis. It is clear that uremia may develop before the kidneys become contracted.

Alterations in the Glomeruli: The glomerulus is nearly always involved, even in the earliest stages of renal amyloidosis. In our 65 cases there was only one instance in which the glomeruli were unaffected and in this there was only a minimal deposit in the medulla. The disturbances of renal function are caused chiefly by obstruction of the glomerular capillaries.

In the older literature the opinion was frequently expressed that amyloid is deposited in the capillary wall. Hueter,¹⁸ in 1908, one of the first investigators to demonstrate the capillary basement membrane, observed that the first deposits of amyloid are on the inner surface of this structure. Ohmori,¹⁹ in 1921, came to the same conclusion. My observations are in full accord with those of Hueter and Ohmori. In sections stained with azocarmine the first accumulations of amyloid are readily seen on the inner surface of the basement membrane, *i.e.*, in the lumen of the capillary (Figs. 1 and 2). At no time is amyloid found external to a demonstrable basement membrane. In the early stages it is usually possible to demonstrate the membrane distinctly from the amyloid, but frequently the amyloid blends with the membrane from the first (Fig. 3). When the capillary is well filled the distinction between membrane and amyloid is usually lost (Fig. 4), but sometimes the membrane is demonstrable in capillaries distended with amyloid (Fig. 5).

An increase of endothelial nuclei in amyloid disease was noted by Hueter, and Fahr also observed this change in some instances. In our material there is a convincing increase of capillary endothelial cells in over 50 per cent of the kidneys. This occurs in capillaries without amyloid, as well as those that contain amyloid. It is also found frequently in kidneys of persons dead of chronic infectious diseases in which there was no renal amyloidosis. Severe infections of various kinds, notably subacute bacterial endocarditis, usually cause an increase of glomerular endothelium (Bell²⁰). This endothelial proliferation is interpreted as acute glomerulitis. It is often quite prominent in amyloid disease (Fig. 1), but in no instance did it reach the degree characteristic of clinical acute glomerulonephritis.

It is obvious that endothelial proliferation is caused by the associated infection and not by the amyloid deposit.

The first accumulation of amyloid is on the inner surface of the basement membrane. If any endothelial nuclei are present at the site of the amyloid deposit they are usually, but not always, displaced toward the lumen of the capillary (Fig. 2). When the capillary is filled by an accumulation of amyloid on one side, the nuclei of the opposite side are left in contact with the basement membrane. In capillaries greatly distended with amyloid the nuclei are frequently scattered throughout the amyloid deposit (Fig. 5).

Raubitschek¹ noted that amyloid may accumulate without narrowing the lumen of the capillary, and Hueter observed that this was due to a simultaneous enlargement of the capillary which may attain two or three times its normal diameter. Fahr⁵ commented on the remarkable permeability of the glomerulus that may be found in advanced amyloidosis. The enlargement of the individual capillaries explains the markedly enlarged glomeruli that are frequently seen. In such large glomeruli there may be a good capillary circulation, the blood being in direct contact with the amyloid deposit. Frequently glomeruli of normal size are seen in which all the capillaries are filled with amyloid—in these no stage of enlargement has occurred. Rarely all the glomeruli are of this type. Ultimately the glomerular circulation is completely blocked by the amyloid deposit, and atrophy of the associated tubule begins. The completely obstructed glomerulus gradually shrinks in size and loses the capacity to react to the specific amyloid stain. Finally it has a homogeneous structure and gives no amyloid reaction (Fahr). Our observations agree with those of Hueter that the glomerular epithelium is largely desquamated in advanced amyloid disease of the glomerulus.

The hyaline glomeruli of the amyloid kidney differ fundamentally from those of chronic glomerulonephritis in that the hyaline is derived from amyloid and not from intracapillary fibers. They also differ sharply from the hyaline glomeruli of primary hypertension which form by fusion of thickened capillary basement membranes.

Casts: Casts play an important rôle in the amyloid kidney. Their prominence usually corresponds to the degree of renal amyloidosis (see Tables II–V). They do not give the amyloid reaction, but apparently are of firmer structure than in other diseases, since they have a much greater tendency to lodge in the tubules. Obstruction

of the lumen causes dilatation and sometimes atrophy of the tubule. Fahr lays great stress on casts as a cause of destruction of the kidney and renal insufficiency. They are often a factor in renal insufficiency and in 1 instance (Table III, Group B, 31-291) they were more important than the glomerular lesion in causing uremia. However, they are seldom the cause of extensive tubular atrophy.

The Arterioles: Hueter thought that the arterioles were frequently involved before the glomeruli, but in our material the deposit seems to occur at about the same time in arterioles and glomeruli (Tables II-V). The amyloid accumulates in the media of the arterioles between the individual muscle cells (Raubitschek). In this respect it differs clearly from the subintimal hyaline deposit found in hypertension. An early amyloid involvement of an arteriole is readily distinguished from hypertension, but a massive amyloid deposit may obscure the evidences of hypertension. The involvement of the arterioles is often a very prominent feature in advanced amyloid disease (Noble and Major¹⁷). There is some narrowing of the lumens of the diseased arterioles, and there is presumably a loss of vasomotor responses. No doubt arteriolar disease is a factor in the destruction of the kidney.

The Medulla: One of the most frequent sites of the amyloid deposit is the medulla (Tables II-V). This seems to take place at about the same time as the deposit in the glomeruli and arterioles. The amyloid accumulates chiefly in the walls of the small vasa recta, displacing the muscular layer. Occasionally there is an accumulation under the basement membrane of the straight tubules or in the interstitial connective tissue. Rarely there is sufficient amyloid in areas of the medulla to compress the tubules and contribute to tubular atrophy.

The Tubules: In two instances in this series the cortical tubules were extensively destroyed by deposits of amyloid under the basement membrane, but this feature was unimportant in the rest. Fahr attaches great importance to tubular degeneration in the destruction of the amyloid kidney, although he concedes that the glomeruli are the chief cause of tubular atrophy. He believes that the tubules may be destroyed by distention from casts and by hyaline granular degeneration. He describes necrotic tubules. In well fixed material we have never observed necrotic tubular epithelium. Hyaline granular degeneration was seen in about half the kidneys of Groups A, B and

C, and in about one-third of those of Group D. This type of degeneration does not seem to progress as far as necrosis of the cell. Fahr also observed that hyaline granular degeneration was less frequent in uremic amyloid kidneys.

Tubular atrophy is usually conspicuous in advanced amyloid disease. The atrophic tubules are nearly always associated with glomeruli obstructed by amyloid. Rarely the atrophy is to be attributed to obstruction by casts or by amyloid in the medulla, and rarely to direct compression of the tubule by amyloid.

The Relation of Amyloid Renal Disease to Glomerulonephritis: In the older literature, before the different types of nephritis were sharply defined, the prevailing opinion was that amyloid disease is not a special type of nephritis but a complication of some form of pre-existing renal disease. Raubitschek¹ stated that in a group of 72 cases of renal amyloidosis there were only 13 examples of pure amyloid disease without inflammatory changes, and that in 1 instance an acute nephritis was present. Hueter¹⁸ stated that a nephritis usually precedes amyloidosis of the kidneys. MacCallum²¹ seems still to hold this opinion, since he states that amyloid is only an incidental deposit which may modify the course of a nephritis but does not cause it.

In the more recent literature the amyloid kidney is usually interpreted as a primary renal disease, but a few authors interpret it as a complication of a preëxistent lipoid nephrosis or glomerulonephritis. Fahr²² classifies amyloid disease as a special form of nephrosis. He recognized a definite increase of endothelial nuclei in 10 of 40 cases, a pronounced proliferation in 2 of these; but he considers the endothelial proliferation a secondary phenomenon and not evidence of a true glomerulonephritis. The disease is classified with the nephroses because the lesions are all interpreted as degenerative and not inflammatory in nature. Fahr recognizes the close clinical resemblance of some cases of amyloid disease to lipoid nephrosis, but he apparently is not convinced that amyloid is superimposed on lipoid nephrosis.

It is frequently difficult to distinguish amyloid renal disease clinically from glomerulonephritis. Some cases resemble the azotemic type, others the hydropic form of glomerulonephritis (lipoid nephrosis). This similarity is evident in a number of recent reports.

Bannick and Barker studied a case of Hodgkin's disease in a male

38 years of age. There was a marked albuminuria and at one time a mild hematuria. The blood pressure was 100/70 to 115/75. There was no edema. There was some impairment of renal function: phenolsulphonephthalein 15 per cent in one hour, blood urea 58 mg. The total serum protein was 3.8 gm., of which 27 per cent was albumin. Cholesterol was 235 mg. The diagnosis of amyloid disease was established by the Congo red test.

Lemierre and colleagues described a case of renal amyloidosis resulting from pulmonary tuberculosis. Albuminuria was present during a period of two years, but there was never any edema. The last determination of serum proteins was globulin 3.19 mg., albumin 2.45 mg. Blood urea was 30 to 38 mg. until toward the end of life when it rose to 124, 237 and 369 mg. The blood pressure was always low.

Linder, Maxwell and Green reported an example of amyloid renal disease in a boy 13 years of age. A mastoid operation at the age of 4 years was followed by the formation of a sinus which discharged from time to time. In 1925 edema and proteinuria were noted. Oct. 29, 1925, he had albuminuria, general anasarca, and blood pressure 112/72. The edema disappeared in December, 1925 and did not recur. The blood urea rose to 199 mg. Blood pressure never increased. The serum proteins varied from 3.8 to 4.7 gm. The cholesterol was 920 mg. in May 1926, but it decreased during the uremia. Death occurred in August, 1926.

Danisch¹⁶ reported an example of amyloid disease in a man 65 years of age. Swelling of the feet was first noticed in 1917. In the fall of 1921 the swelling increased. He was confined to bed from January to March, 1922. In August, 1923, he had heavy albuminuria, very marked edema, and a blood pressure of 173/101. The blood pressure remained high. In December, 1924, the non-protein nitrogen was 141.3 mg. Death occurred in December, 1924. Contracted amyloid kidneys were found postmortem.

In several of our cases the resemblance to chronic glomerulonephritis is obvious. No. 31-1934, Group C, was regarded clinically as the hydropic type of glomerulonephritis until the Congo red test was made. The associated chronic arthritis, of course, suggested the possibility of amyloid disease. The same clinical diagnosis was made in No. 32-175, Group D. No. 28-64, Group D, was diagnosed clinically as the azotemic form of chronic glomerulonephritis. Amyloid disease was not suspected since there was no associated infection.

When an infectious process of long duration is present, one should think first of amyloid disease as an explanation of renal symptoms. When there is no known cause for amyloidosis the true nature of the renal lesion may not be suspected. An enlarged firm liver suggests amyloid. The Congo red test is of great value in many instances.

Amyloidosis is a special type of renal disease. In some instances it exhibits the clinical features considered characteristic of nephrosis and in others it presents the phenomena regarded as typical of nephritis. Occasionally the disease progresses from a picture of nephrosis to one of nephritis. It does not clarify our conceptions to force it into the group of nephroses, especially since the distinction between nephrosis and nephritis is becoming less sharp as our knowledge of renal disease increases. It is true that the renal lesions are chiefly degenerative in character, but there is commonly a definite endothelial proliferation at the onset of the disease.

Amyloidosis is a primary disease of the kidney in the sense that it is rarely superimposed on any clinical form of renal disease. At the onset there is commonly a mild acute diffuse proliferative glomerulitis caused by the infection responsible for the amyloidosis, but this inflammatory reaction does not attain the intensity characteristic of clinical acute glomerulonephritis, and it is frequently absent entirely. We have one example of amyloidosis superimposed on a hypertensive kidney, but the renal disturbance in this instance was largely caused by the amyloid deposit. A combination of amyloid with chronic glomerulonephritis seems possible, but we have not seen such a case.

SUMMARY

A study of 65 cases of amyloid disease of the kidneys is reported. These are arranged in four groups corresponding roughly with the degree of glomerular involvement.

In Groups A and B the symptoms are essentially those of the underlying infection, and with few exceptions albuminuria or edema is the only symptom referable to the kidneys.

In Group C there is some impairment of renal function, and in Group D there is evidence of advanced renal insufficiency.

Albuminuria is rarely absent, but the amount of albumin does not indicate accurately the extent of the amyloid deposit.

Edema is a variable feature with no evident relation to the degree of renal damage.

Hypertension is occasionally found in amyloid disease with renal insufficiency. It is probably due to obstruction in the arteriolar and glomerular circulation.

Renal insufficiency is a frequent cause of death. It is caused chiefly by amyloid deposits in the glomerular capillaries, but obstruction of the tubules by casts and amyloid deposits in the medulla, around the tubules and in the arterioles, are often important factors in the production of uremia.

In the glomerulus amyloid is deposited on the inner surface of the capillary basement membrane. Endothelial nuclei are frequently displaced inwardly and become scattered through the amyloid. The capillaries usually become greatly distended with amyloid and they may remain permeable in the presence of massive deposits. The glomerular epithelium degenerates and is desquamated.

There is commonly a definite increase of endothelial nuclei in the glomerular capillaries preceding the deposit of amyloid. This is attributed to the underlying infection. It is not sufficiently prominent to be identified with clinical acute glomerulonephritis.

Amyloidosis is a special form of renal disease. There is no advantage in classifying it as a nephrosis. A sharp distinction between nephrosis and nephritis has not been established.

Amyloidosis is a primary renal disease. It is rarely a complication of a preëxistent clinical renal lesion.

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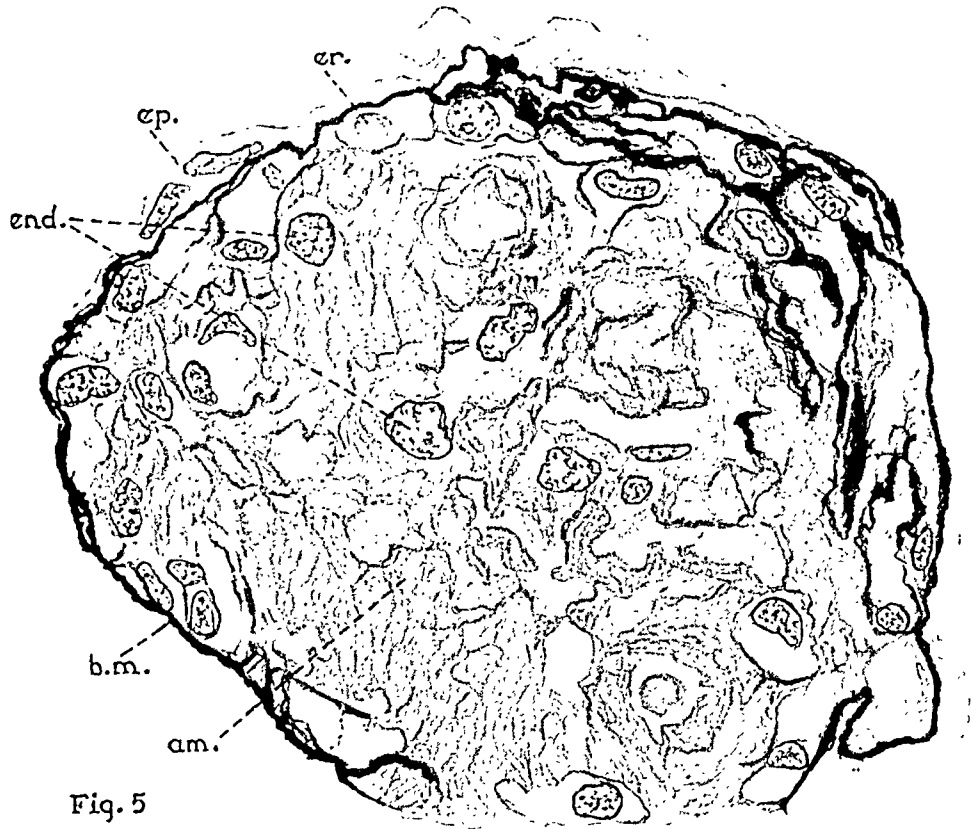
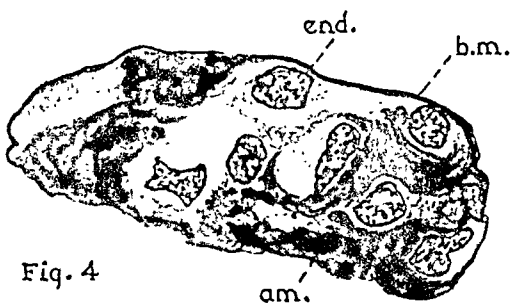
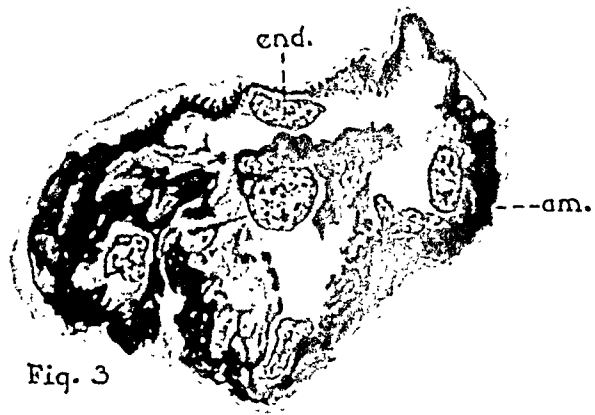
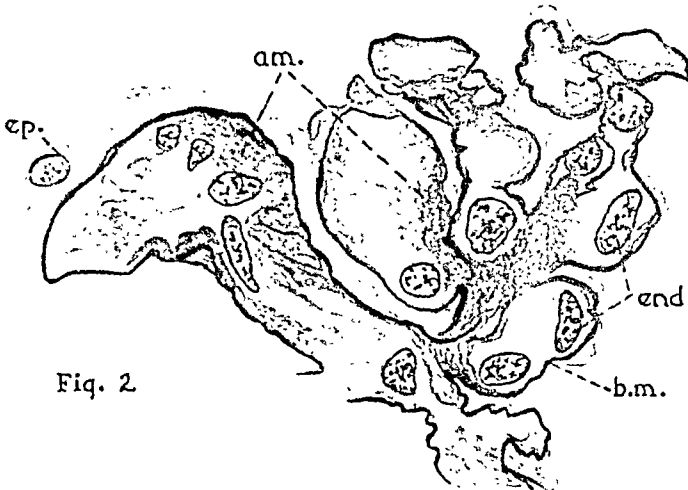
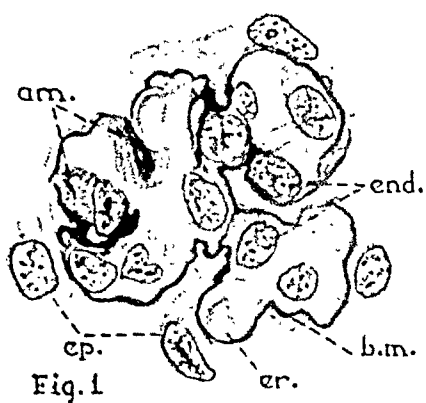
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DESCRIPTION OF PLATE

PLATE 30

The drawings were all made from preparations stained with azocarmine (Mallory-Heidenhain stain). Each represents a small portion of a glomerulus: am. = amyloid deposit, b.m. = basement membrane, end. = endothelial nucleus, ep. = glomerular epithelial cell, er. = erythrocyte. $\times 1000$.

- FIG. 1. Capillaries showing the initial stage of amyloid disease. The amyloid is deposited on the inner surface of the basement membrane. The membrane is indistinct under one of the amyloid masses. There is a marked increase of endothelial cells.
- FIG. 2. Capillaries showing a moderate amount of amyloid. The basement membrane is everywhere distinct. There is an increased number of endothelial nuclei, and some of them are displaced centrally. The epithelial cells are largely desquamated.
- FIG. 3. Capillary illustrating a type in which the initial deposit of amyloid blends with the basement membrane.
- FIG. 4. Capillary containing a large amyloid deposit. Only a small lumen persists. The basement membrane is no longer demonstrable under the larger amyloid masses. There is a definite increase of endothelial nuclei, and some of them are still in contact with the basement membrane.
- FIG. 5. Capillary greatly distended with amyloid. The basement membrane is distinct. Small spaces persist through which the blood still circulates. There is an increased number of endothelial nuclei. This type of capillary is found in the very large glomeruli.



THE RELATION BETWEEN THE MITOCHONDRIA AND GLUCOSE-GLYCOGEN EQUILIBRIUM IN THE LIVER *

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In recent years considerable work has been undertaken in an effort to discover the rôle of the mitochondria (chondriosomes) of the hepatic cell, especially in relation to the glycogen cycle. This relationship has been variously interpreted by different investigators. The reason for the wide variation in interpretation is, no doubt, largely due to the fact that the function of these very minute bodies must of necessity be interpreted in terms of changes in morphology and distribution within the cell. Obviously such interpretations are little more than conjectures unless they are supported at every stage by experimental data.

The literature on the subject of mitochondria is so vast that we shall refer only to papers that deal directly with the relation of mitochondria to the glucose-glycogen cycle. Arnold ¹ in 1908 found hypertrophy of mitochondria under certain conditions. He concluded that this increase in size is directly proportional to the amount of glycogen in the liver and assumed that the swelling of the chondriosomes is due to glycogen accumulating in them in the form of drops (Tropfen). Bang and Sjövall,² and Noël ³ state that the distribution of glycogen within the cytoplasm of the cell does not conform to the distribution of the mitochondria. Noël, after careful and extensive study, concluded that the cells from the morphological standpoint appeared to be the seat of two separate or independent processes, "les processus mitochondriaux et le processus glycogénique." The location of the glycogen and the chondriosomes, glycogen in spaces limited by the protoplasmic trabeculations, and chondriosomes in the trabeculations, makes it difficult to understand that there is any connection between the two. Mann ⁴ in 1928 reviewed the subject of mitochondria in glycogen relationship and concluded that none exists.

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Kater⁵ has recently pointed out that all observers heretofore had attempted to correlate mitochondrial morphology with static aspects of glycogen deposition and suggested the desirability of investigating the morphology of the mitochondria of the hepatic cell at a time when the glycogen-glucose equilibrium is known to be disturbed.

Kater's choice of a plan of experiment was rather unfortunate in so far as a test of the conditions he had in mind are concerned. No determinations were made, either chemical or histological, of the glycogen content of the livers, so that it was impossible to know in which direction or in what degree the glycogen synthesis-glycogen hydrolysis equilibrium was disturbed. Blood sugar determinations which were carried out are of little value in such a study because marked increases and decreases may occur without any change in the liver glycogen, or, as in the case of epinephrine, a high blood sugar may be accompanied by an increase in liver glycogen. Kater's animals were fasted for twenty-four hours, which would reduce the liver glycogen to a very low value and make variations in the equilibrium difficult to study. Some animals were kept under ether anesthesia for a long time, a condition under which the hepatic glycogen changes would at most be uncertain. He gave some animals insulin. Its action on glycogenolysis is very indefinite, although in time it definitely reduces liver glycogen. Epinephrine, which was also used to modify the equilibrium, has a variable action causing early increases and later decreases in glycogenolysis. In any case a knowledge of the time factor is necessary for the interpretation of the results.

Contrary to the results of most of the other investigators cited, Kater concluded that some relation exists between the mitochondria of the hepatic cell and the glucose-glycogen equilibrium. He found in cases of increased metabolic activity a marked tendency toward enspherulation and hypertrophy of mitochondria.

Since Kater's paper was published two of his pupils, Clark and Hair,⁶ have reported their studies on the mitochondria of the hepatic cell of the frog in normal and hyperglycemic states. These authors believe that much of the variation of opinion on the subject at hand is due to failure of the various investigators to take cognizance of two phases of hepatic cell activity, namely, carbohydrate metabolism and bile secretion. They point out that bile secretion is closely related to the presence of food in the intestine and believe, therefore, that it is necessary to disturb the mechanism through

some other means than feeding in order to cause activity in only one phase.

These considerations led them to choose the frog in its dormant state as their experimental animal. Besides a control group of ten animals, three other groups were used as follows: one group was injected intramuscularly with adrenalin-hydrochloride solution, another group was given glucose, and a third group was etherized for two and one-half hours.

The authors found no disturbance of the morphology of the mitochondria obtained from the sugar injections. Adrenalin-hydrochloride injections and etherization caused increase in size and enspheralization of mitochondria, most marked about the central vein. They agree with Kater that there is some relation between the mitochondrial morphology of the hepatic cell and the glucose-glycogen equilibrium.

While the frog in its dormant state may present some advantages over other laboratory animals in a study of this character, it must be borne in mind that the mitochondria of the liver cell are extremely variable in morphology, as the authors themselves found in a study of their ten control animals. It should also be remembered that glycogen synthesis in the liver is far more active at night while no food is being ingested than it is during the daytime. It seems probable that bile secretion interferes but slightly with glycogen metabolism.

MATERIAL AND METHODS

Experiment 1. Carrot-Feeding Experiments: The remarkable deposition of glycogen in the liver, which we found to follow carrot feeding of rabbits,⁷ first interested us in the problem being considered here. In view of Kater's work it seemed desirable to correlate this increase with the mitochondrial changes. Three groups, each composed of three young female rabbits, were placed upon diets of dried alfalfa, dried carrot and fresh carrot⁷ respectively. Twelve days later and 8 hours after being fed, when they were full of freshly ingested food material (rabbits eat more or less steadily throughout this period), all of the rabbits were removed from their diets and one from each group killed immediately. Twelve hours later a second was sacrificed and after 24 hours the third of each group was killed. The chemical analyses are presented in Table I and depicted graphically in Text-Fig. 1.

The animals were killed by injecting 5-10 cc. of air into the marginal ear vein. Before either respiratory or cardiac movements had entirely ceased the liver was rapidly excised and samples simultaneously removed for chemical glycogen determinations and for fixation in the various solutions for mitochondria and glycogen stains. Thirty seconds sufficed for all of this. Great care was exercised in cutting and handling small bits of tissue for fixation because of the marked changes in the finer cell morphology associated with squeezing, drying, and so on. Samples for the glycogen determinations were weighed quickly to within 0.5 per cent and dropped into boiling 60 per cent KOH and the glycogen analyzed by a modification of Pflueger's method, determining the glycogen as glucose after acid hydrolysis in place of weighing the substance.

HISTOLOGICAL METHODS

In recent years a number of relatively simple methods for staining mitochondria have been worked out. We have used two of these methods with some success.

Small blocks of tissue taken with great care as described above were fixed in Regaud's formalin and bichromate mixture. Other blocks were fixed in Flemming's fluid in preparation for Benda's crystal violet alizarin method. Satisfactory results were not obtained by this method. Using the blocks fixed in formalin and potassium bichromate (Regaud) the iron hematoxylin method of Heidenhain gave a fairly good differentiation. Good results were also obtained by use of Cowdry's modification of Altmann's anilin fuchsin and methyl green method, employing the same fixation. This stain gave a slightly better differentiation than the iron hematoxylin method. By this method the mitochondria stain brilliant red while the nuclei are olive green.

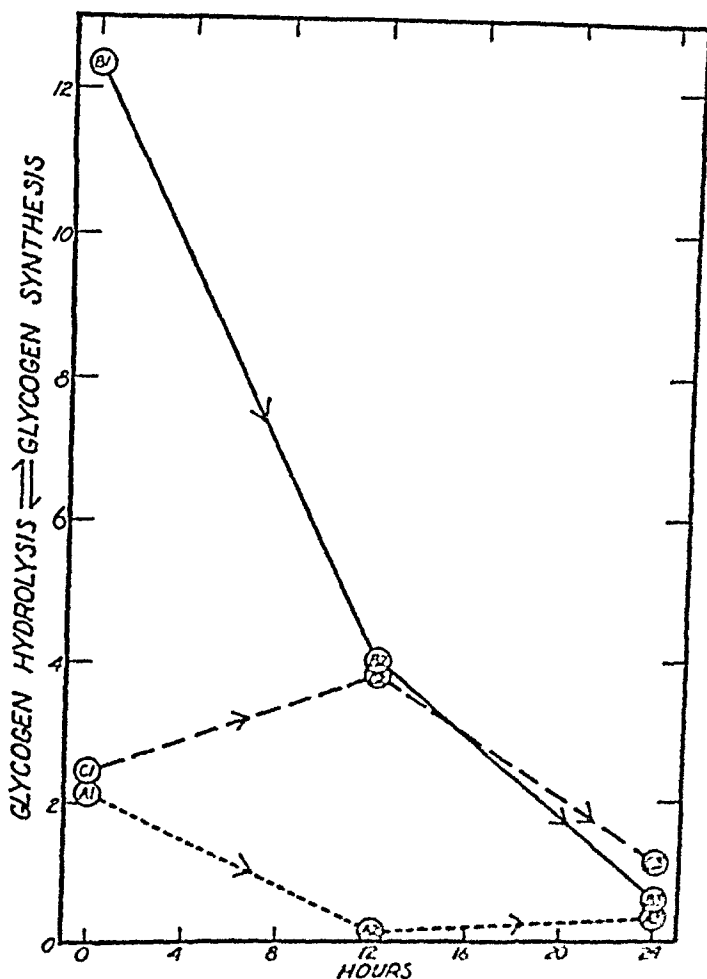
Small blocks, only 3-4 mm. square and about 2 mm. thick, were embedded in hard paraffin (56-58° C). Sections were cut 2 and 5 microns thick. The mitochondria were too dense for good differentiation in most of the 5 micron sections. Those cut at 2 microns were quite satisfactory and stained well.

Other small blocks of liver tissue were also taken for fixation in 95 per cent alcohol. These were embedded in celloidin and stained by Best's carmine method to show the relative amounts of glycogen present and its distribution within the liver lobules.

RESULTS

The results of Experiments 1, 2 and 3 are statistically and graphically shown in Tables I to III.

Experiment 1 (Table I): The effects of heavy carrot feeding on hepatic glycogen and mitochondria were studied in this experiment.



TEXT-FIG. 1. Graph (Experiment 1) showing changes in the percentage of hepatic glycogen plotted against time in hours under the various experimental conditions. Arrows directed away from the base line indicate synthesis of glycogen; those directed toward the base line indicate hydrolysis of glycogen.

Rabbit A1 fed on an ordinary diet of alfalfa served as a control. In the control the hepatic glycogen by chemical determination was slightly over 2 per cent. Sections stained by Best's carmine method show the cells of approximately the central two-thirds of the lobules filled with red granules. In Table I this amount is designated as

++. It will be seen, from a study of the various tables, that each plus sign, in a general way, corresponds to approximately 1 per cent glycogen by chemical determination. The histological examinations for glycogen were made independently of the figures obtained for the chemical determinations. With one or two exceptions the agreement between the two methods is very close. The diagrammatic drawing of the liver cell (A₁, Table I) shows that the mitochondria in the control liver are tiny bacilliform rods scattered indiscriminately throughout the cytoplasm (Fig. 1). Reference to Tables II and III shows the mitochondria of the other control animals very similar in morphology and distribution.

Rabbits A₂ and A₃, fasted for 12 and 24 hours respectively, showed marked reductions in the glycogen content of the livers by both chemical and histological methods. The mitochondria of the liver cells exhibit some tendency to become spherical or coccoid in form.










The changes produced in the liver cells of Rabbit B₁, fed on pulverized dried carrot, are the most outstanding of any in our series in regard to both glycogen content and transformation of mitochondria. The liver glycogen reached the astounding figure of 13.10 per cent, while every cell of the liver lobules is completely filled with coarse, red-stained granules of glycogen. In some of these cells the granules are so coarse that only 10-20 angular masses of glycogen can be counted in a single cell. The average is probably 30-35, while in the sections of the control liver (A₁) two to three times as many fine granules are present in each of the cells about the central veins. Corresponding with this great storage of glycogen profound changes are observed in the cells stained for mitochondria. The hepatic cells are markedly swollen and clear, except about the nuclei and cell peripheries where dark masses of mitochondria have accumulated (Fig. 2). In many cells the nucleus is almost obscured by the condensation of mitochondria upon it. Likewise scattered clumps of two or three up to a dozen or more mitochondria are piled up on the limiting membrane of the cell. The masses of mitochondria, especially about the nuclei, show as irregular black smudges (Fig. 2), so dense that individual morphology can be made out only in occasional ones at the outer edges of the masses. These, together with the few scattered mitochondria within the clear zone of cytoplasm, show that the rods are plumper than in the control; a few are elongated and filamentous (some cells show filaments reaching from the nuclear

mass to the cell boundary); others are diplo- or coccoid in form. No differences of any note are observed between cells in the central and peripheral portions of the lobules.

In our carrot-feeding experiments⁷ conducted a year ago the same swollen, clear cells with vacuolated cytoplasm were observed

TABLE I

Experiment 1. Effects of Carrot-Feeding on Liver Glycogen and Mitochondria

Rabbit No.	Diet	Conditions	Body weight	Liver weight	Liver glycogen	Histological glycogen	Mitochondria
			gm.	gm.	per cent		
A1	Alfalfa	No food withdrawal	1670	52	2.12	++	
A2	"	12 hrs. after food	1740	36	0.12	sl. tr.	
A3	"	24 hrs. after food	2080	47	0.26	tr.	
B1	Dried carrots	No food withdrawal	1860	58	13.10	++++...+	
B2	"	12 hrs. after food	2030	76	3.96	++++	
B3	"	24 hrs. after food	2030	50	0.55	+ (-)	
C1	Fresh carrots	No food withdrawal	2095	55	2.47	++	
C2	"	12 hrs. after food	1960	58	3.77	+++	
C3	"	24 hrs. after food	2240	65	1.08	+	

in the livers of these rabbits. At that time Best's carmine stain revealed huge quantities of glycogen and the "vacuoles" in the cells were found to correspond closely to the coarse clumps of glycogen seen in the Best's carmine preparations.

A second (B₁) animal fed on dried carrots in the same manner as the first gave similar results. The glycogen content of the liver reached 11.6 per cent, while the Best's carmine preparation showed every liver cell loaded with coarse clumps of glycogen. The mitochondrial stains by the iron hematoxylin and acid fuchsin-methyl green methods reveal almost identically the same morphology and paranuclear condensation of mitochondria as have been described.

After one of the carrot-fed animals (B₂) had fasted for 12 hours the chemical glycogen was reduced to 3.96 per cent. Glycogen is still abundant in the Best's carmine preparations, but some of the cells in the peripheries of the lobules are slightly pale. The liver cells are not so uniformly loaded as in B₁ and the granules not quite so coarse. Several counts indicate an approximate average of 35-50 granules per cell.

The mitochondria, as may be seen in the diagram, are quite uniformly distributed in the cytoplasm, in some cells moderately more abundant about the nucleus, while nearly all have assumed a coarsely granular or coccoid form. Many of the larger spheres are so perfectly round they give the impression of having a fluid or semi-fluid content.




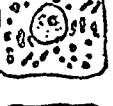

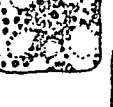
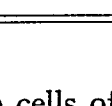
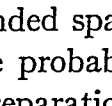

A third animal of the group fed dried carrot (B₃) after fasting 24 hours, showed a liver nearly depleted of glycogen (0.55 per cent). Histologically only occasional small groups of liver cells contain red granules. Stains for mitochondria show a distribution and morphology indistinguishable from the control (A₁).

Two additional animals fed on a dried carrot diet, treated as the B₂ and B₃ animals respectively, gave similar results.

A group of three rabbits was fed on fresh carrots and alfalfa for a period of 12 days. These animals were treated in the same way as the foregoing group fed on dried carrots. The results may be seen in the table. Due, apparently, to the great amounts of water and roughage in the fresh carrots, rabbits are unable to consume sufficient bulk to raise the glycogen content of their livers to any marked degree. Rabbit C₁ produced 2.47 per cent glycogen in the liver, while C₂ showed 3.77 per cent after a 12 hour fast. This increase may be accounted for on the basis of the slowness of digestion of bulky food such as fresh carrots, and on individual differences between the two animals. After a 24 hour fast Rabbit C₃ had only 1.08 per cent glycogen in the liver.

TABLE II

Experiment 2. Effects of Food Withdrawal and Glucose Feeding on the Liver Glycogen and Mitochondria

Rabbit No.	Conditions	Body weight	Liver weight	Liver glycogen	Histological glycogen	Mitochondria
		gm.	gm.	per cent		
A ₁	No withdrawal of food	3210	95	1.59	+	
A ₂	No withdrawal of food	3030	69	0.73	+ (-)	
B ₁	24 hrs. without food	1890	62	0.50	+ (-)	
B ₂	24 hrs. without food	1960	61	0.78	+ (-)	
C ₁	As B, then 5 hrs. at 0°C	2740	72	0.75	+ (-)	
C ₂	As B, then 4 hrs. at 0°C	1990	50	0.70	+ (-)	
D ₁	As A, then 15 gm. glucose and killed in 3 hrs.	2970	80	2.70	++	
D ₂	As A, then 15 gm. glucose and killed in 3 hrs.	3430	104	5.70	++	
E ₁	As B, then 15 gm. glucose and killed in 3 hrs.	1760	72	4.63	+++	
E ₂	As B, then 15 gm. glucose and killed in 3 hrs.	1760	48	3.82	+++	
F ₁	{ As B, then 15 gm. glucose at 0 and 3 hrs. and killed 6 hrs. after first dose.	2320	96	3.50	+++	
F ₂		2440	91	4.17	+++	

The mitochondria show no marked changes in the cells of these livers. Quite a few granules or coccoid forms are intermixed with the rods, the former being predominant. Clear, rounded spaces in the cytoplasm of the cells in Rabbits C₂ and C₃ are probably fat globules. These are present in the Best's carmine preparations, as well as in the sections stained by the iron hematoxylin method.

The graph, Text-Fig. 1, shows at a glance the changes in glycogen content of the various livers of the rabbits in Experiment 1, together with the relative rapidity of the glycogenolysis. The arrows directed away from the base line indicate glycogen synthesis, while those pointing toward the base line signify glycogenolysis.

Experiment 2 (Table II): This experiment was designed to demonstrate the various effects of food withdrawal and glucose feeding on hepatic glycogen and mitochondria. The two control animals, A₁ and A₂, showed some variation in the glycogen content of the livers, but the mitochondria are practically identical with those seen in the control of Experiment 1. Animals B₁ and B₂ following a fast of 24 hours show little change from the controls. To our surprise, 4 hours at 0° C. following a 24 hour fast failed to reduce the hepatic glycogen of Rabbits C₁ and C₂ below that of the two previous animals. The mitochondria show only some increase in the relative numbers of granular forms.

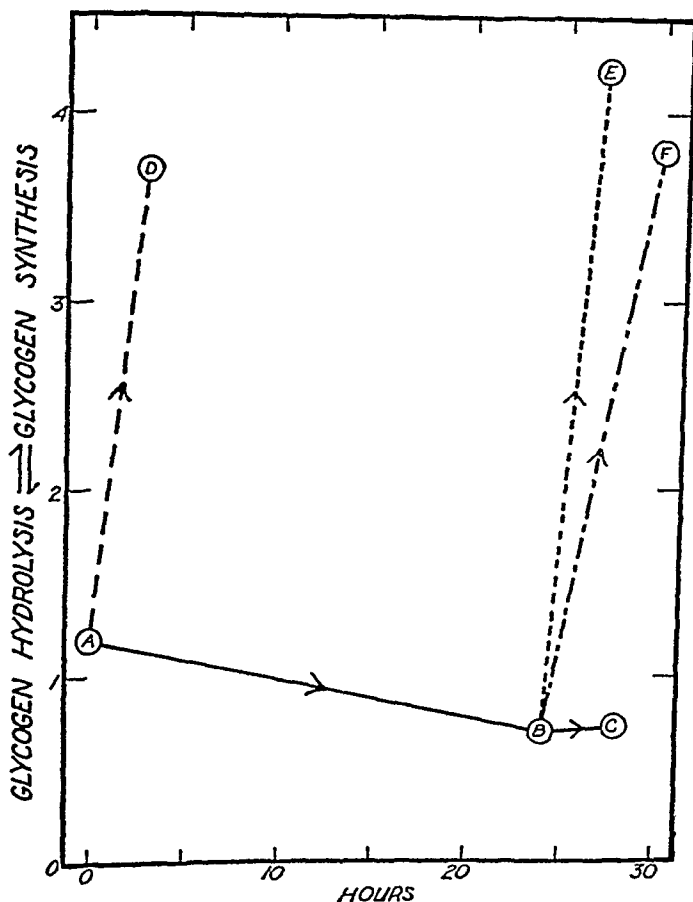
Glucose fed by stomach tube in animals D₁, D₂, E₁, F₁, and F₂ brought about rapid storage of glycogen in the liver. Whether the animals are first fasted for 24 hours as in E₁ and E₂ makes no difference in the final result. There is some discrepancy between the figure of 5.70 per cent glycogen in Rabbit D₂ and only ++ glycogen as determined by the histological method. The latter determination checks with that of the D₁ liver, while the chemical determination is almost twice as great. We are unable to explain this discrepancy.

The mitochondria in the glucose-fed animals appear somewhat plumper than in the foregoing, with a greater tendency to assume the spherical form. Figure 3 represents a camera lucida drawing of three cells from F₁ drawn under the same magnification as Figures 1 and 2. It may be seen that mitochondria are accumulated quite definitely about the nuclei and somewhat on the limiting cell membrane as well. Nearly all are rather coarse spherules, some of them larger than in Figure 2. Numerous clear spaces in the cytoplasm are probably the spaces occupied by fat globules.

The graph, Text-Fig. 2, which for obvious reasons is not drawn to the same scale as Text-Fig. 1, shows, in the main, rapid synthesis of glycogen.

Experiment 3 (Table III): This experiment was designed to show the effects of food withdrawal plus adrenalin on liver glycogen and mitochondria. Rabbits were fasted for 24 hours and then given

1 mg. of adrenalin intravenously, except the first two animals A₁ and A₂ which served as controls. Sets of two animals were sacrificed after 1, 2 and 3 hours respectively. This experiment shows that adrenalin injections after fasting first deplete the liver of glycogen during the first and second hours. By the end of 3 hours, however,



TEXT-FIG. 2. Graph (Experiment 2) showing changes in percentage of hepatic glycogen plotted against time in hours. Each letter represents the average figure for two animals. Text-Figs. 1 and 2 are drawn to different scales.








the hepatic glycogen has again returned to a normal figure (1.44 per cent).

Mitochondria may undergo, according to Cowdry, three kinds of changes, namely, qualitative, quantitative, and changes in distribution. Kater⁵ found in his experiments with cats a tendency for the rods and filaments in the liver cells to take the form of granules and spherules when the glucose-glycogen equilibrium was disturbed. As we have stated before, he was dealing with very small quantities of glycogen in the individual liver cells.

Our experiments show minimal changes in the mitochondria in Experiment 3, where the quantities of glycogen are small. This experiment duplicates in part Kater's experiments with adrenalin, except that we have used rabbits instead of cats.

TABLE III

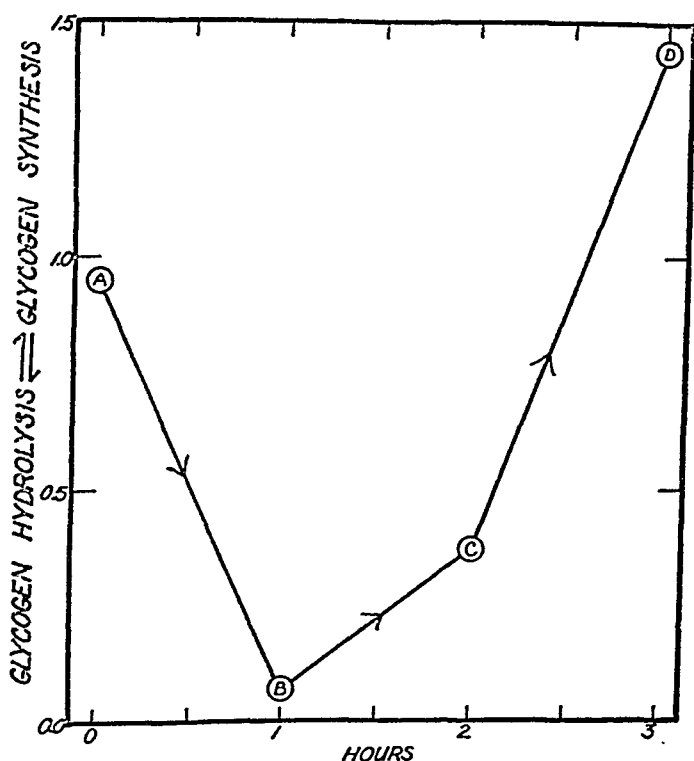
Experiment 3. Effects of Food Withdrawal Plus Adrenalin on Liver Glycogen and Mitochondria

Rabbit No.	Time after injection of adrenalin	Body weight	Liver weight	Liver glycogen	Histological glycogen	Mitochondria
A1	No adrenalin (control)	gm. 1350	gm. 42	per cent 0.97	(+ -)	
A2	No adrenalin (control)	1690	48	0.92	+	
B1	1 hour	1450	41	0.09	tr.	
B2	1 hour	1640	49	0.12	tr.	
C1	2 hours	1370	49	0.52	+ (-)	
C2	2 hours	1350	51	0.19	sl. tr.	
D1	3 hours	1580	44	1.44	+	

During the progress of an earlier experimental study in carrot-feeding⁷ we discovered that the liver cells became loaded with glycogen beyond anything we had ever seen. It occurred to us that this would be an ideal condition in which to study mitochondria in relation to the glucose-glycogen equilibrium. If the chondriosomes take any part in this process it might be expected that relatively greater changes would be observed in them while the cell is under the strain of storing an abnormally high content of glycogen. Our

hopes have been fully realized, as can be seen by the bizarre changes wrought in the mitochondria of Rabbit B₁ (Experiment 1), Figure 2.

Our experiments show a marked disturbance in the mitochondria of the hepatic cell during excessive glycogen storage and during subsequent hydrolysis. The most outstanding change is due to their redistribution within the cell, especially their condensation about the nucleus. Changes in morphology consist in hypertrophy,



TEXT-FIG. 3. Graph (Experiment 3) showing changes in percentage of hepatic glycogen plotted against time in hours. A, B and C each represents the average for two animals, D for one animal only. Scale not the same as in Text-Figs. 1 and 2.

filament-production and enspherulation. Large granules which appear to be semifluid are evident during rapid glycogenolysis.

The mitochondria show a moderate tendency to form fairly coarse spherules. In the liver of Rabbit D₁ a condition is reached approaching that of F₁, Experiment 2 (Fig. 3), rather closely.

The glycogen lysis and synthesis curves of Experiment 3 are illustrated in Text-Fig. 3. Here again the scale is not the same as in Text-Figs. 1 or 2.

COMMENT

Several investigators (Bang and Sjövall,² and Noël³) have claimed that no demonstrable relation exists between glycogen deposition and changes in the mitochondria. Altmann⁸ as early as 1889 observed that the granules seen in the hyaloplasm varied in size and in disposition with the particular stage of digestion. Mann⁴ states that under normal physiological activity changes in shape are usual. Imbibition of known substances from the cytoplasm by the mitochondria is well established but uncertainty exists as to what processes are involved. He further states that actual absorption probably occurs, accompanied by chemical dissociation and synthesis.

Since it is established that mitochondria of the stomach, pancreas and liver change their shape during digestion it would seem to indicate that they play some part in this important process. Cowdry⁹ states in his review that the morphology of mitochondria in these organs is alike in nearly related animals. He believes that "this constancy in shape where function is similar indicates that the morphology of mitochondria is a fundamental property ingrained in the organization of the cell and that it is not always a passing trivial affair which varies from moment to moment." He further states that changes in shape of mitochondria constitute by far the most delicate criterion of many types of cell injury at our disposal.

It is not improbable that the peculiar paranuclear distribution of mitochondria at the height of glycogen storage in Rabbit B₁ may be largely a mechanical effect — the mitochondria being pushed aside by the formation of coarse clumps of glycogen within the cytoplasm. The consistency of the nuclear accumulations, whether the nucleus is in the center or at one side or one end of the cell, suggests that the process is not wholly mechanical. Some chemotaxis appears to exist between nucleus and mitochondria. Uniform transformation of filamentous and rod-like mitochondria into coarse spherules during rapid glycogenolysis (Rabbit B₂, Experiment 1) can be accounted for since this is a usual type of transformation observed in the mitochondria of various species of animals and plants. On the purely physical side it would appear that this process of hypertrophy and enspherulation may be the result of imbibition of fluid at the time that hydrolysis of glycogen is very active. Noël³ speaks of the "granules of coque" which are mitochondria containing fat globules.

Recently, Kater and Smith ¹⁰ have shown that fat globules develop within mitochondria of the hepatic cell in rats following the feeding of cane sugar. The observations of these authors indicate that actual synthesis of fat takes place within the mitochondria.

SUMMARY AND CONCLUSIONS

1. Disturbances of the glucose-glycogen equilibrium in the livers of rabbits have been produced by feeding large quantities of dried and fresh carrots followed by periods of fasting; by feeding glucose to fasting animals; and by injections of adrenalin in fasting animals.

2. Excessive amounts of glycogen, as high as 13.1 per cent, were obtained in the animals fed on dried carrots. Amounts ranging from 3.5 to 5.7 per cent were obtained in the animals fed fresh carrots and in those given glucose. Injections of adrenalin produced mainly glycogenolysis.

3. Marked changes in the mitochondria were found in the animals fed on dried carrots. In place of the usual short bacilliform rods, long filaments, coarse spherules and plump rods were found condensed about the nucleus and to a lesser degree about the cell membrane.

4. Twelve hours fasting in this group produced coarse spherules without definite arrangement in the cytoplasm. Many of these appear to be semifluid.

5. Administration of glucose caused hypertrophy and enspherulation of mitochondria with some tendency to paranuclear arrangement.

6. We conclude that some relation exists between the mitochondria of the hepatic cell and the glucose-glycogen equilibrium. Whether or not the chondriosomes act as catalysts, as they appear to do in the synthesis of fat within the hepatic cell, we are unable to say.

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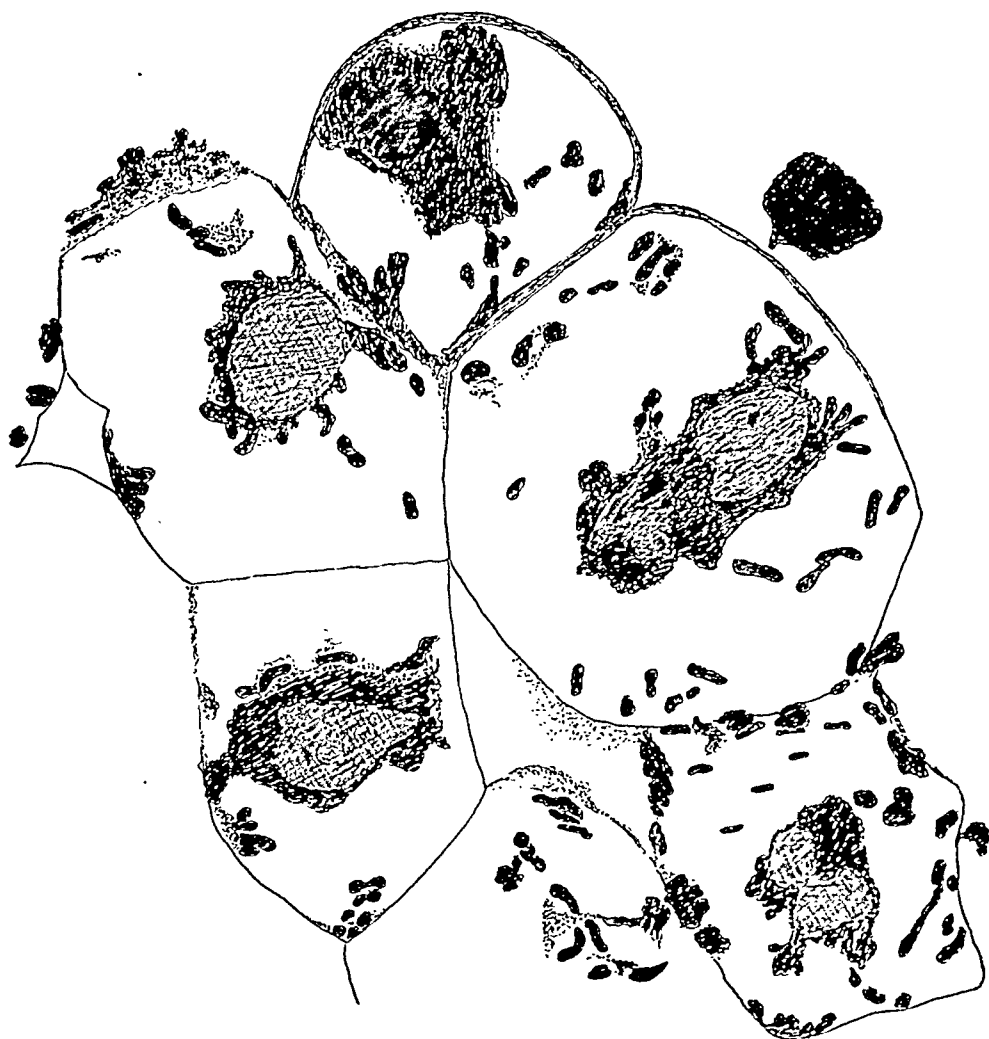
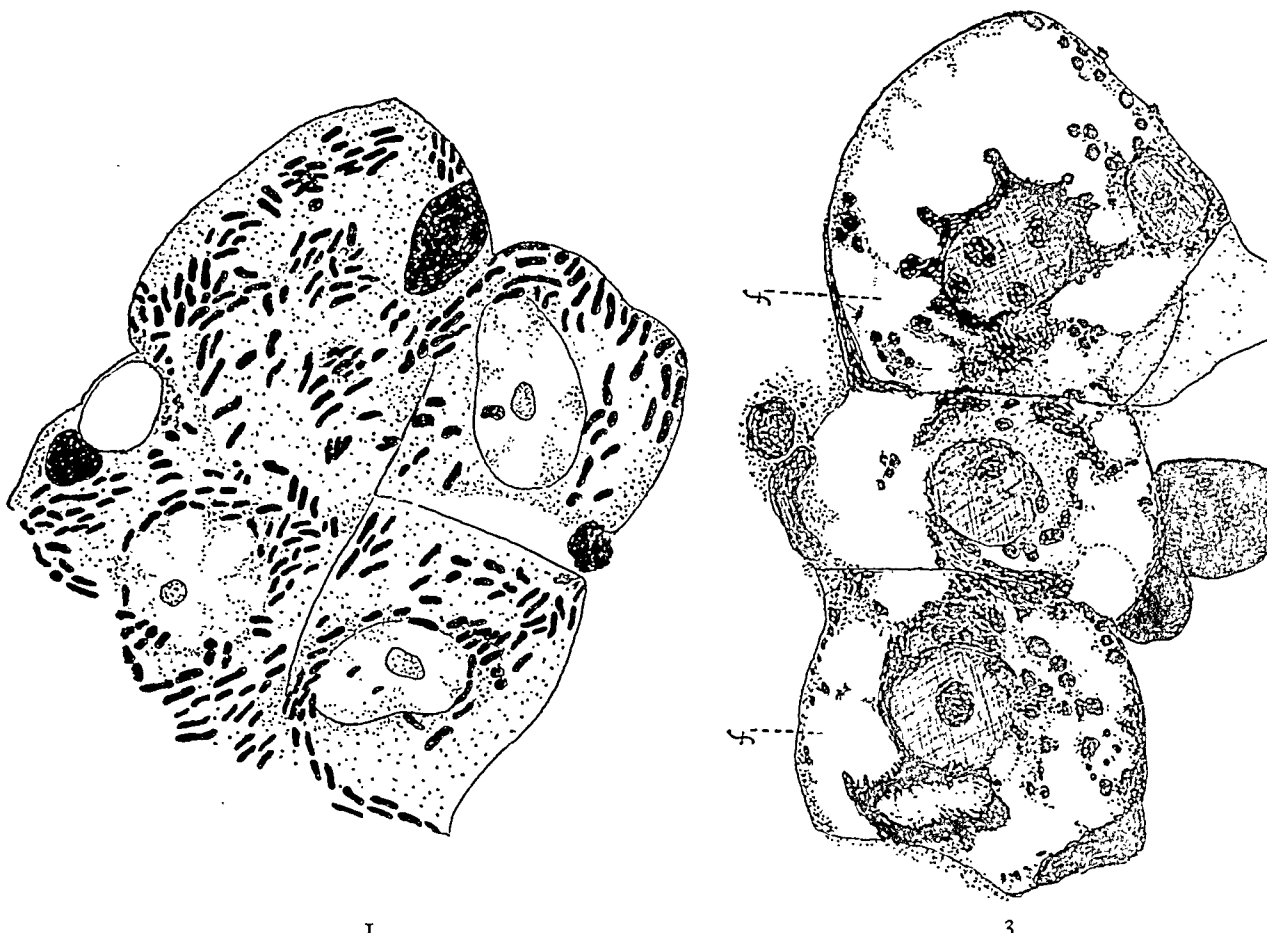
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DESCRIPTION OF PLATE

PLATE 31

- FIG. 1. (Experiment 1, Rabbit A1.) Drawing of several liver cells from control, showing the usual type of rod-like mitochondria. The solid black bodies are red blood cells. (Camera lucida, Zeiss 2 mm. obj., 15 × ocular.)
- FIG. 2. (Experiment 1, Rabbit B1.) Drawing of a group of liver cells under the same magnification as Fig. 1, showing swelling of the cells, clear cytoplasm, hypertrophied and filamentous mitochondria with accumulation about the nuclei.
- FIG. 3. (Experiment 2, Rabbit F1.) Drawing of three liver cells under the same magnification as Figs. 1 and 2, showing hypertrophy and enspherulation of mitochondria with tendency to accumulate about the nuclei. Clear spaces (f) in the cytoplasm are apparently due to fatty droplets.



THE SIMILARITY OF VIRUS PNEUMONIA IN ANIMALS TO EPIDEMIC INFLUENZA AND INTERSTITIAL BRONCHO- PNEUMONIA IN MAN *

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A characteristic and unique type of pneumonia frequently complicates certain epidemic diseases. This condition which MacCallum¹ has styled interstitial bronchopneumonia is observed most frequently in fatal cases of measles, whooping cough and epidemic influenza. During an influenza epidemic or following an outburst of measles the pathologist suddenly encounters many such cases, but even under ordinary circumstances in every large hospital with a pediatric service he is kept aware of the condition by deaths from whooping cough and measles. Although the pathological anatomy of interstitial bronchopneumonia was described clearly many years ago by Bartels² in Germany, and by Delafield³ in this country, it has become increasingly familiar since the World War through the publications of MacCallum,⁴ who studied the pneumonias associated with the epidemics of measles in the United States Army camps, and those of Opie and his associates,⁵ who concerned themselves with postinfluenzal pneumonia.

The gross lesions of interstitial bronchopneumonia can be observed best on a smoothly cut surface of the lung, which is often studded with small nodules of grayish yellow tissue that sheath the branching bronchioles. A thick exudate usually can be squeezed from the central bronchioles, which generally show thickened walls and stand out from the surface as rigid tubes. These yellow peribronchiolar nodules have a striking gross resemblance to tubercles or foci of tuberculous bronchopneumonia, as pointed out long ago by Honl.⁶ Although usually small, they may develop large enough to become confluent, but even when they coalesce the presence of the thickened bronchial walls, often pink due to granulation tissue, suffices to prevent one from confusing the consolidated areas with other types of pneumonia. The intervening lung tissue is usually

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collapsed and often dark red, but it is not apt to show hemorrhage or edema, except in individuals dying at the height of an influenza epidemic.

Under the microscope the central bronchioles are plugged with granular leukocytes, between and sometimes within which bacteria of various types are found. The most striking feature, however, is the great thickening of the bronchial wall and of the alveolar walls in an encircling zone of lung tissue. This thickening is due mostly to an infiltration with large mononuclear cells, lymphocytes, plasma cells and proliferating fibroblasts. The consolidated alveoli are often filled exclusively with large mononuclear cells, but in places they are occupied by tangled masses of dense, red-staining fibrin in which there is an occasional wandering cell. Of course, many alveoli contain polymorphonuclear leukocytes as well as red blood corpuscles, and this is especially true in material from the influenza epidemic of 1918 in which the presence of a variety of bacteria complicates the picture. In many cases of measles or whooping cough, however, the polymorphonuclear cells are scarce and often confined to the bronchioles. The lymphatics of the septa and bronchial walls, together with those about the blood vessels, are dilated and filled with inflammatory cells mixed perhaps with fibrin and, at times, bacteria. These are briefly the important characteristics of interstitial bronchopneumonia that distinguish it from those of the ordinary broncho- or lobular pneumonia,* which so frequently develop terminally in all types of illnesses, and in which some bronchioles and their connected alveoli are filled with a uniform exudate made up of polymorphonuclear leukocytes, fibrin, red cells and bacteria without the cellular infiltration of the interstitial tissue.

The conclusions we have reached have been based upon the study of autopsy material and upon the results of experimental infection in animals. We have examined the sections from 50 autopsies upon patients who died of epidemic influenza during the 1918-19 pan-

* The terms bronchopneumonia and lobular pneumonia are used interchangeably to designate a patchy consolidation of the lung. Each term leaves much to be desired since all acute pneumonias involve the bronchi, and the areas of consolidation referred to are never strictly lobular. We shall use the term lobular pneumonia except when the patchy consolidation is accompanied by a cellular infiltration of the interstitial tissue, in which case MacCallum's recently designated term "interstitial bronchopneumonia" will be employed.

demic. In addition we have studied 25 autopsied cases of measles, 40 of whooping cough and 100 others as controls. The majority of the latter were from children who had died of diseases other than those forming the basis of this report.

EXPERIMENTAL PROCEDURES

While studying vaccine virus pneumonia in rabbits ⁷ we were impressed by the similarity of the lesions to those found in interstitial pneumonia as well as in the hemorrhagic and edematous type of consolidation observed in the lungs of individuals dying a few days after the onset of epidemic influenza. In this work bacteriologically sterile vaccine virus (neurovirus of Levaditi) was injected into the lungs through the trachea, and the animals were killed from 1 to 5 days later. In the majority of the animals no bacteria could be demonstrated in the lungs. However, two animals not included in our published report contracted a pulmonary bacterial infection which complicated the experimentally produced virus pneumonia. Both of these animals developed a pleurisy with a thin, turbid brown fluid. Cultures showed the presence of a streptococcus in pure culture in one animal, and mixed with staphylococci and colon bacilli in the other. The lesions in these two animals so closely resembled the changes found in certain cases of epidemic influenza that the experimental use of a combination of virus and bacteria was at once suggested. The interval between the injection of bacteria and virus was varied in different series of experiments. In one series bacteria were mixed with the virus before inoculation and the mixture injected into the trachea. In other experiments diluted virus was injected first and followed from 1 to 7 days later, in different series, by the injection of a suspension of bacteria. Animals were killed at intervals varying from 1 to 18 days after the first inoculation of virus.

The virus was prepared by diluting with Locke's solution an emulsified rabbit testicle 4 days after intratesticular inoculation with the neurovirus of Levaditi. In experiments in which virus alone was used 1 to 2 cc. of a 1:10 dilution were injected. When bacteria were also injected a 1:10 dilution proved too strong, so that 1 to 2 cc. of a 1:100 dilution were usually employed in the latter experiments.

Three types of bacteria were used. One was a hemolytic streptococcus isolated from a spontaneous infection in a rabbit. Another was a staphylococcus cultured from the lung of a child with post-pertussis interstitial bronchopneumonia, and the third organism was a stock strain of *B. pertussis*. These organisms were grown on blood agar slants from which thin suspensions were prepared in salt solution.

Individual protocols will not be given, for over 100 animals have been used in these studies and the crucial experiments have been repeated often enough with sufficient controls to eliminate fortuitous reactions.

Vaccine Virus Pneumonia

In another place⁷ we have described in detail the pulmonary changes induced by vaccine virus without bacteria, and here only the more important lesions will be discussed. Twenty-four hours after the injection of virus there is a marked tracheitis and bronchitis with a red, swollen mucosa. The lungs show elevated patches of translucent gelatinous consolidation, some of which become hemorrhagic after the second day, producing firm, dark red to purple-colored areas. About the third or fourth day, on a cut surface of the lung in an involved area the bronchi are prominent due to thickening of the walls. In an occasional animal this thickening and infiltration extend beyond the bronchioles to produce small yellowish peribronchiolar nodules resembling tubercles, which are very conspicuous where the lung tissue forming the background is hemorrhagic. The bronchial lymph nodes are enlarged and soft.

Microscopic study shows the areas of gelatinous consolidation, the earliest gross lesion, to consist of groups of alveoli filled with coagulated edematous fluid and varying amounts of fibrin. At this stage the alveolar walls show no structural change, although their capillaries are at times congested. Mononuclear cells may escape into the alveoli in small numbers. In later stages this lesion may resolve or it may progress in two different ways. In the most acute reactions the alveolar epithelium degenerates and desquamates. Damage to the capillaries causes hemorrhage into the alveoli. As this lesion develops the entire alveolar wall, including the capillary, degenerates and in this way large irregular areas of lung tissue, although retaining their structural outline, become completely ne-

crotic, resembling the center of an infarct. This necrosis appears to be due to the direct action of the virus upon the tissue and is not the result of thrombosis of blood vessels. If the animal survives great numbers of polymorphonuclear leukocytes invade the necrotic tissue. We suggest that this acute process be called "hemorrhagic virus pneumonia." Instead of this hemorrhagic and necrotizing process a proliferative cellular lesion may develop. When this takes place the bronchioles and the alveolar walls become thickened by an infiltration with mononuclear cells. The infiltration is most marked in the alveoli close to a bronchiole or in the neighborhood of a blood vessel, the adventitia of which is thickened and infiltrated. The cells of the alveolar epithelium enlarge and the great frequency of mitotic figures clearly indicates their active multiplication. These large mononuclear cells become more numerous in the alveoli and in some they displace all other elements. It is proposed that this proliferative reaction be termed "interstitial virus pneumonia." Although these two lesions are almost always distinct and separate, nevertheless, elements of each can occasionally be recognized in one lesion.

These two types of lesion are not regarded as different stages of the same process but rather as quantitative reactions to different concentrations of virus. A large quantity of strong virus injected into the lungs of a normal animal tends always to produce hemorrhagic virus pneumonia with extensive necrosis, a minimal cellular proliferation and early death. A small quantity of it, or a more dilute virus, as a rule calls forth the proliferative cellular lesions of interstitial virus pneumonia, which is not immediately fatal. A moderate amount of virus introduced into the lungs of a vaccine-immune animal, or the injection of virus mixed with immune serum into a normal animal, results in a proliferative lesion, if there is any reaction at all.

The mucosa of the larger bronchi is hyperemic, but the epithelial cells are usually not destroyed. In the bronchioles of an involved area, however, a patch of necrotic epithelium is frequently seen with collections of polymorphonuclear leukocytes about the dead cells. Cytoplasmic inclusions (Guarnieri bodies) are found in the bronchial epithelium in about 10 per cent of non-immune animals.

The perivascular lymphatics, as well as the lymphatics of the septa and those of the bronchial walls, become greatly distended and

often filled with thrombus-like coagula of albuminous fluid and fibrin, in the meshes of which large mononuclear wandering cells, leukocytes and lymphocytes are entangled. The walls of the adjacent blood vessels become edematous and the connective tissue and muscle fibers are pushed apart. Foci of necrosis appear in the adventitia and may extend into the media. When this occurs large numbers of polymorphonuclear leukocytes and a few mononuclear cells infiltrate the vessel wall.

It must be emphasized that all these lesions occurred in the absence of bacteria. Repeated cultures of lung tissue remained sterile, and bacteria were never found in the many sections of the most severe lesions stained by various methods for the demonstration of bacteria in tissues.

Virus Pneumonia with Superimposed Bacterial Infection

With the exception of typical lobar pneumonia, which is notoriously difficult to induce experimentally, we have been able to reproduce by the use of vaccine virus and bacteria practically every pulmonary lesion that has been described as a complication of influenza or measles. The effect of the bacteria alone was first studied. When injected into the lungs through the trachea the animals responded differently to each of the three strains of bacteria used. The only reaction noticed after injection of large quantities of *B. pertussis* was a mild inflammation of the tracheal and bronchial mucosa. Pneumonia was never observed. Because of its apparent non-virulent character this old stock strain of the organism was used in only a few experiments after vaccine virus and in each instance the virus pneumonia was not altered in any remarkable manner.

The streptococcus isolated from a rabbit regularly produced an ordinary lobular pneumonia with a typical exudate of polymorphonuclear leukocytes in bronchioles and a group of connecting alveoli. Even in large numbers this organism seldom proved fatal. The staphylococcus recovered from the lungs of a child dying of pertussis proved to be the most virulent organism. Small numbers of this coccus incited a lobular pneumonia, but a larger number (one-half of an agar slant) produced death in 24 to 48 hours from septicemia. It was with these last two mentioned organisms that most

of the experiments with combined injections of virus and bacteria were carried out.

It will be recalled that vaccine virus alone produces two distinct types of pneumonia. One is an acute hemorrhagic and edematous consolidation with foci of necrosis, and the other an interstitial cellular infiltration with large mononuclear cells, fluid and fibrin in some of the alveoli.

The important lesions or complications superimposed on virus pneumonia by the simultaneous or subsequent introduction of bacteria may be classified as follows: (a) acute bronchitis and lobular or bronchopneumonia; (b) abscess formation; (c) pleurisy and empyema; and (d) bronchiectasis, squamous cell "metaplasia" of bronchial epithelium and organizing pneumonia.

Acute Bronchitis: It has already been pointed out that the characteristic cellular exudate of pure vaccine virus pneumonia is composed principally of large mononuclear wandering cells and that polymorphonuclear leukocytes appear in large numbers only after the virus has caused necrosis of the exudate or of the pulmonary tissue when the granular leukocytes infiltrate the dead tissue. Within 24 hours after the introduction of bacteria into the lungs of an animal, which already has a virus pneumonia, a typical pyogenic exudate composed of polymorphonuclear leukocytes, bacteria and a scant amount of fibrin appears in the terminal bronchioles and in a varying number of connected alveoli. In some cases the bacteria are found only in the bronchioles and do not reach the alveoli. Frequently the bacteria reach parts of the lung that have not previously been affected by the virus, and in such places there is a pure lobular pneumonia.

Abscess Formation: Pulmonary abscesses, both solitary and multiple, occurred in many of the animals that survived the injection of bacteria for a week or 10 days. The abscesses were most frequently situated in the lower lobes posteriorly and were often close to the pleural surface. Some of the abscesses had an opaque, soft yellow center occasionally surrounded by a narrow zone of hemorrhage; others had a softened center and an ill-defined edge that fused with a flabby, consolidated peripheral zone of lung tissue, with or without hemorrhage. Microscopically some of these irregular abscesses appear to have been produced by the bacteria spreading rapidly from the central bronchiole throughout an area that already

had been rendered necrotic by the action of virus. The center of such an abscess is a formless mass of necrotic tissue and bacteria, but toward the periphery there is often a zone of necrotic lung tissue resembling in every respect the necrosis observed in pure virus pneumonia. Bacterial stains seldom reveal any bacteria in the outer part of this necrotic edge. If one is unfamiliar with virus pneumonia it would be natural to ascribe the peripheral necrotic zone to the diffusion of bacterial toxins into the surrounding lung tissue from the center of the abscess. Of course all the abscesses may originally have started in damaged tissue, for it is well known that lung abscesses cannot readily be produced by injecting ordinary pyogenic bacteria into a normal lung. However, it seems likely that in some instances an abscess started at the center of a large area of virus necrosis and the animal died before the bacterial infection involved the entire necrotic area.

Pleurisy and Empyema: A serofibrinous pleurisy was found in two animals that contracted a spontaneous bacterial infection after the injection of vaccine virus. In one animal the pleurisy was bilateral and in the other the right side only was affected. The fluid was thin and light brown in color with numerous shreds and flakes of fibrin that settled to the bottom. A streptococcus was cultured from this fluid. Microscopically these lungs show a widespread hemorrhagic virus pneumonia with patches of necrosis that involve practically the entire lower lobes. In addition there are areas of lobular pneumonia, and where these extend to the pleural surface it is covered with a layer of fibrin. Streptococci can be found everywhere in the necrotic lung tissue, usually without any reaction about them. There are no abscesses.

Patches of fibrinous exudate were often encountered upon the pleura of animals that developed lobular pneumonia after the injection of bacteria wherever the areas of consolidation reached the surface of the lung. Occasionally, when such patches were numerous a few cc. of cloudy or thick yellow fluid were found in the pleural cavity. Therefore no sharp distinction could be drawn between serofibrinous and purulent pleurisy.

Empyema has occurred three times in animals surviving the injection of bacteria for 7 to 10 days. The only essential difference between the three cases concerned the amount of pus in the pleural cavities. In every instance the empyema was accompanied by a

subpleural abscess that had ruptured into the pleural cavity. The animal killed 10 days after the injection of bacteria had an abscess in the upper part of the right lower lobe, which had ruptured into the fissure, thus causing an interlobar empyema. Thick, creamy yellow pus extended through the fissure and out into the pleural cavity posteriorly where there was a large encapsulated empyema pocket extending throughout the entire length of the thoracic cavity. Adhesions between lung and chest wall along the axillary line prevented the pus from reaching the anterior portion of the pleural cavity.

Microscopically the lungs show a well advanced virus pneumonia with marked cellular infiltration of the interstitial tissue. Many of the alveoli contain mononuclear cells and dense masses of fibrin. There is also a superimposed purulent bronchitis and a lobular or bronchopneumonia with abscess formation.

In pure virus pneumonia the lymphatics of an involved area are widely distended with albuminous fluid, fibrin, and in places with groups of inflammatory cells, principally large mononuclear wandering cells. Many of the animals that developed pleurisy and empyema had masses of bacteria in these dilated lymphatics. In some sections the subpleural lymphatics contained many organisms. Although the granulocytes appeared to be more numerous in these infected lymphatics than in the same channels in pure virus pneumonia, they were not so abundant as might be expected in the presence of so many bacteria. It is obvious that bacteria can spread more rapidly throughout a lung that already has a well developed virus pneumonia, by way of the dilated lymphatics, than they can through a normal lung in which the lymphatic channels have not previously been altered. In the control animals, which had received only bacteria, this rapid dissemination by way of lymphatics was never observed. Thus it appears that a virus pneumonia induces such changes in the lung that bacteria, which in normal animals can incite only a limited infection, are enabled to multiply and spread throughout it causing extensive and fatal lesions. The same bacteria are incapable of producing such lesions without the preliminary action of the virus upon the pulmonary tissue. At least two lesions of virus pneumonia are conducive to the more rapid growth and dissemination of bacteria. The foci of pulmonary necrosis support a more luxuriant bacterial growth than normal tissue will allow, and

the wide dilatation of the lymphatic channels enhances the opportunities for the more rapid distribution of organisms throughout the lung.

Bronchiectasis and Associated Lesions: A slight dilatation of the smaller bronchi was frequently observed in animals that developed severe purulent bronchitis or abscesses. One of the animals with empyema showed marked distention of the bronchi in the lower lobes. The bronchi were uniformly distended to about twice their normal diameter, and in a few places localized, spherical bronchiectatic cavities were formed. A thick mucopurulent fluid filled the bronchial tree, and after the exudate was washed away the mucosa was found to be of a deep red color, due to hyperemia and petechial hemorrhages. Shreds of fibrin were attached to the epithelium in a few places.

The epithelial lining of the bronchiectatic cavities is in places completely destroyed; elsewhere only parts of it are necrotic and the dead tissue is usually covered with a layer of fibrin and leukocytes. Regeneration of the epithelial cells has resulted, in a few areas, in the appearance of flattened cells arranged in several layers resembling squamous epithelium. This change is identical with the condition described by many authors as squamous cell metaplasia.

In some bronchi, especially in those in which there is extensive destruction of the mucosa, inflammatory cells infiltrate the entire wall. Polymorphonuclear leukocytes are found in greatest abundance near the exposed surface, and mononuclear cells, lymphocytes and plasma cells deeper in the wall. The alveoli surrounding such bronchi usually contain masses of fibrin or a cellular exudate composed of a few mononuclear cells and many granular leukocytes. In the most chronic bronchiectatic lesions fibroblasts, and at times newly formed capillaries, can be found in the bronchial walls.

In one instance an organizing pneumonia was found in an animal with bronchiectasis. A cellular fibrous tissue replaced the exudate in a group of alveoli about dilated bronchi. This loose fibrous tissue in many places extended by means of narrow processes through openings between neighboring alveoli. Capillaries were never found in the fibrous tissue within the alveoli.

THE ETIOLOGY OF EPIDEMIC INFLUENZA

Despite the voluminous literature on influenza and the bewildering number of clinical and experimental investigations carried on during and since the 1918 pandemic, there is still no unanimity of opinion concerning its etiology. Indeed there is a difference of opinion as to what lesions constitute influenza, *per se*, and what should be regarded as complications. In general the prevailing ideas regarding the causative agent are represented by one of the two following conceptions: (a) influenza is due to the bacillus influenza of Pfeiffer, (b) the etiological agent of influenza is a filter-passing virus.

The Rôle of B. Influenzae

Many investigators still regard the bacillus of Pfeiffer as the etiological agent of influenza because it so frequently has been found somewhere in the respiratory tract of individuals ill with this disease. In one investigation at Camp Pike, Opie, Blake, Small and Rivers⁵ found *B. influenzae* "invariably present in all cases of influenza." However, they discovered that in normal groups the incidence of the same organism varied between 11 and 88 per cent, and that about 80 per cent of individuals with measles also harbored this bacillus. Park⁸ found it in 80 per cent of cases. Wolbach,⁹ Pritchett and Stillman,¹⁰ and Spooner, Scott and Heath¹¹ all found the bacillus frequently. In England, McIntosh¹² found the bacillus in 42 out of 69 cases, and other English reports also show a lower incidence of the bacillus than our own. Messerschmidt, Hundeshagen and Scheer¹³ in Germany report the presence of the bacillus in 90 per cent of individuals affected during the height of the epidemic. On the other hand, the Camp Lewis Pneumonia Unit,¹⁴ Kinsella,¹⁵ Hirsch and McKinney,¹⁶ as well as some investigators in England and Germany, found *B. influenzae* rather infrequently. However, in spite of these negative results there can be little doubt that *B. influenzae* is present in the majority of individuals suffering from epidemic influenza.

The influenza bacillus is frequently found in the nasopharynx of healthy individuals, although during epidemic periods its incidence increases. To cite only one example, Williams¹⁷ and her coworkers found 40 per cent of normal persons as well as 92 per cent of those

ill with influenza harboring the bacillus. They also observed an increase in the frequency of pneumococcus and of hemolytic streptococcus as well as *B. influenzae* in patients suffering from influenza.

B. Influenzae is found associated with a variety of other conditions. Mention has already been made of its presence in 80 per cent of patients with measles at Camp Pike. These cases occurred during the influenza epidemic, but Wollstein,¹⁸ Davis,¹⁹ Liebscher²⁰ and others have found it associated with measles during interepidemic periods, and Boggs²¹ and Lord²² report its frequency in a variety of acute and chronic diseases of the respiratory tract. The bacillus has also frequently been isolated from children suffering from whooping cough.

Opinions concerning the etiological rôle of the influenza bacillus are as divergent as the above reports of its incidence in the disease. The Medical Research Committee of Great Britain²³ were of the opinion that the orthodox conception that Pfeiffer's bacillus is the cause of epidemic influenza is no longer tenable. MacCallum²⁴ and Kinsella¹⁵ regarded the organism as a secondary invader. MacCallum, however, thought that *B. influenzae* was the cause of a purulent bronchitis and a lobular or bronchopneumonia complicating some cases of influenza. Opie *et al*⁵ say: "The constant association of *B. influenzae* with influenza suggests that it is the cause of the disease." He adds, however: "It is possible that *B. influenzae* is a secondary invader, entering the respiratory tract when susceptibility is increased by an unknown virus causing influenza."

In order to reconcile the prevalence of *B. influenzae* in the nasopharynx of healthy individuals, with the view that this bacillus is the cause of influenza, those who champion this conception claim that *B. influenzae* suddenly undergoes a rapid increase in virulence, changing from a harmless saprophyte to a highly virulent pathogenic organism. In spite of the well known fact that the virulence of an organism can be enhanced by repeated passages from host to host, such an extreme increase in virulence as would be necessary to uphold this view finds no support in our knowledge of the increase and decrease of bacterial virulence in epidemic diseases caused by microorganisms.

Of all the experimental efforts to reproduce influenza with cultures of *B. influenzae* those of Opie and coworkers,⁵ performed at Camp Pike during the epidemic in the fall of 1918, were done under

especially favorable circumstances. Suspensions of recently isolated cultures were used. Although these caused a mild, self-limited respiratory illness, somewhat similar to slight attacks of influenza in man, it required the addition of virulent streptococci or pneumococci to provoke severe pulmonary lesions; but these organisms are capable of producing the same changes without the assistance of the influenza bacillus.

By the use of fluid cultures of *B. influenzae* and the peritoneal exudates of infected animals, Blake and Cecil²⁵ were able to initiate an acute respiratory infection similar "in its clinical course, symptoms and complications with influenza." Ten out of twenty-seven animals developed a bronchopneumonia. The chief characteristics of this lesion were hemorrhage and edema, bronchiolitis and peribronchial infiltration. We believe that true interstitial bronchopneumonia was not reproduced. The predominant cells in the exudate and infiltrations appear to have been polymorphonuclear leukocytes. The hemorrhagic and edematous exudates, of course, resembled the early acute lesions of influenza in man. These changes are to be expected with liquid cultures, for in them Parker²⁶ proved the presence of a bacterial toxin. Fildes and McIntosh,²⁷ as well as Huntoon and Hannum,²⁸ were able to produce edema and hemorrhages in the lungs and elsewhere with filtrates of fluid cultures of *B. influenzae*. Winternitz and coworkers²⁹ have shown that similar hemorrhagic and edematous lesions with necrosis of the alveolar epithelium occur after exposure to toxic gases such as chlorine and phosgene, and comment upon their similarity to the lesions found in the acute hemorrhagic stage of influenza in man. Although it seems possible to produce a self-limited respiratory affection somewhat similar to mild cases of human influenza with massive cultures of *B. influenzae*, or even to incite a lobular pneumonia, and with fluid cultures or their filtered toxins to effect hemorrhages and edema in the lungs of animals, no one has yet produced pulmonary changes with this bacillus that simulate those of epidemic influenza in man, or its more important complications. Any adequate explanation of the pathogenesis of influenza must not only consider the acute phases of the disease but must also account for the interstitial bronchopneumonia, as well as for the variety of pulmonary complications that frequently follow.

Evidence for a Filterable Virus

A great deal of experimental work, especially since 1918, supports the conception that influenza is caused by a filter-passing virus. During the second wave of the late pandemic, evidence was brought forth to support this idea by Gibson, Bowman and Connor³⁰ in England, and by Nicolle and Lebailly³¹ of the French army. In 1919 Yamanouchi, Sakakami and Iwashima,³² using filtered and unfiltered samples of sputum from influenza patients, reproduced clinical influenza in 18 out of 24 volunteers by injections into the nose and throat. The remaining 6 had recovered from the spontaneous disease and were therefore immune. Filtered blood produced similar results, but pure cultures of *B. influenzae*, or mixtures of this and other bacteria found in the respiratory tract, failed to induce illness in 14 healthy persons.

All these experiments merely prove the existence of a toxic substance in the sputum of patients suffering from influenza, and are open to the criticism that a living virus was not demonstrated. Passage experiments, if performed, were negative. Carefully controlled experiments have been undertaken by Lister and Taylor,³³ and by McIntosh,¹² and all have given negative results. A survey of the published work upon the relation of a filterable virus to influenza fails to reveal any convincing proof of its presence.

THE LESIONS OF INFLUENZA AND THEIR SIMILARITY TO
THOSE OF VIRUS PNEUMONIA

The earliest acute pulmonary lesion of influenza is a hemorrhagic and edematous lobular consolidation. Areas resembling hemorrhagic infarcts are not uncommon. Microscopically the interlobular septa and the connective tissue about the vessels and bronchi are edematous. Groups of alveoli are filled with coagulated albuminous fluid, red blood cells or fibrin. The appearance of the alveolar contents varies. In some the edematous material is granular, in others a clear, pink-staining colloid-like mass is seen. Varying amounts of fibrin and red cells may be found in the alveolar coagulum. Occasionally dense masses of red blood cells fill a group of alveoli and make it impossible to distinguish the alveolar walls traversing the area.

Necrosis of the epithelium of the bronchioles and alveoli is frequent and in places this necrosis is not confined to the epithelial

layer but extends into the walls of bronchioles and alveoli. In this manner areas of lung tissue are killed *en masse*, producing foci of necrosis.

This early hemorrhagic lesion found a few days after the onset of the disease has been emphasized by Wolbach,⁹ Winternitz *et al*,²⁹ and Goodpasture and Burnett.³⁴ The latter authors point out the scarcity of bacteria at this stage and suggest that the injury may be due to some toxic agent acting upon the lung tissue. Winternitz and coworkers remark about the similarity of the lesions to those occurring after exposure to irritating gases.

In a short time other lesions appear, usually from 5 to 10 days or later after the onset of the disease. These may be described briefly as a cellular infiltration of the interstitial tissue, a non-purulent alveolar exudate and lobular or bronchopneumonia. Taken together these constitute interstitial bronchopneumonia.* Lobar pneumonia, pleurisy, empyema, bronchiectasis, organizing pneumonia and other lesions may also follow.

The edematous and hemorrhagic consolidation with occasional foci of necrosis found in the lungs of animals after the injection of strong vaccine virus closely resembles the early lesions found in epidemic influenza. The paucity of bacteria in the latter is significant, since the experimental virus lesions are bacteriologically sterile. The only elements of interstitial bronchopneumonia found in simple virus pneumonia are the interstitial cellular infiltration, the mononuclear exudate and the fibrin masses in the alveoli. The lobular or bronchopneumonic component of interstitial bronchopneumonia, however, may be superimposed upon vaccine virus pneumonia by the intratracheal injection of bacteria that induce a purulent bronchitis and lobular pneumonia with a polymorphonuclear exudate in the bronchioles and connecting alveoli.

* The strict etymological meaning of the term "interstitial bronchopneumonia" probably indicates an interstitial inflammation or pneumonia which is bronchial in distribution, and does not include the lobular or bronchopneumonic element consisting of a polymorphonuclear exudate in the central bronchioles and in groups of adjacent alveoli that usually accompanies the interstitial inflammation. According to this strict interpretation it would be necessary to speak of interstitial and bronchopneumonia in order to include both elements. However, from MacCallum's description of the condition,¹ it is evident that he intended the term "interstitial bronchopneumonia" to connote the polymorphonuclear exudate in the bronchioles and alveoli, as well as other salient features that are not defined by the term "interstitial bronchopneumonia." It is in this broader sense that we use the term.

The more chronic pulmonary and pleural lesions that can be produced by a combination of virus and bacteria have already been described, and here it is only necessary to point out their similarity to many of the various complications that sometimes follow epidemic influenza. Lobar pneumonia is the only common complication of influenza that we have not reproduced, perhaps because the pneumococcus was not used, or because of the type of animal used.

Opie⁵ and coworkers have described an acute endophlebitis that they observed in association with interstitial suppurative pneumonia and in other lesions complicating influenza. This vessel change resembles the vascular lesion we have described in vaccine virus pneumonia. Their similarity can be appreciated by comparing Figure 14 in Opie's book with Figure 5 in our paper on vaccine virus pneumonia.⁷ Although there were bacteria in all the lungs in which Opie observed this endophlebitis, the presence of similar lesions in rabbit lungs free from bacteria indicates that this lesion may also be caused by a filterable virus.

A thin, ribbon-like layer of hyaline material lining alveoli and alveolar ducts is sometimes found in interstitial bronchopneumonia, whether the latter is the result of influenza, measles or whooping cough. Wolbach and Goodpasture regarded this hyaline layer as characteristic of postinfluenzal pneumonia. Although masses of dense fibrin, staining brilliantly with eosin, are commonly found in animals with virus pneumonia, this homogeneous hyaline layer lining the alveoli was observed only a few times in our experimental lesions.

In some cases of influenza complicated by a streptococcus, areas of pulmonary necrosis overgrown with this organism have been observed, and between these foci streptococci have usually been present in smaller numbers. The opinion has frequently been expressed that the necrosis in these foci is due to the localized growth of so many bacteria. Similar foci of necrosis not overgrown with bacteria can be found in the lungs of individuals dying a few days after the onset of influenza, and in our experimental animals massive colonies of bacteria were frequently found at the centers of such necrotic foci, which apparently had been previously produced by virus, while in other well preserved regions of the lung the organisms had not grown so prolifically. Because of these observations it seems probable that, in some cases of influenza at least, these

areas of necrosis may antedate the entrance of the streptococcus and the remarkable growth of the latter in these foci may be the result of the preëxisting necrosis and not its cause.

While the experiments that have been performed with vaccine virus and bacteria in rabbits cannot prove the existence of a virus in epidemic influenza in man, the similarity of the anatomical lesions suggests such a possibility.

THE ETIOLOGY AND ANATOMICAL LESIONS OF MEASLES

Although most workers are now agreed that measles is caused by a filterable virus, a few still present evidence in support of various bacteria. The most suggestive work is that of Tunnicliff and Hoyne,³⁵ who describe a small Gram-positive, filter-passing, green-producing diplococcus as the causative agent. Tunnicliff and her associates have demonstrated antibodies for this organism in the blood of measles patients. Long and Cornwell,³⁶ however, did not succeed in finding organisms of this group in 26 cases of measles; and Bradford³⁷ as well as Smith³⁸ could not distinguish these cocci isolated from measles from similar hemolytic strains obtained from healthy individuals. Conclusive evidence to prove that these cocci are the cause of the disease is wanting. Typical measles has not been produced by the injection of cultures of these organisms into human beings, as is true of the production of the disease by other methods.

The work of Hektoen,³⁹ and of Blake and Trask,⁴⁰ supplies convincing evidence in favor of the theory that measles is due to a filterable virus. Hektoen showed that the disease can be induced in susceptible human beings by injecting the blood of measles patients taken shortly after the rash appears. Blake and Trask were able to reproduce a condition very similar to human measles in monkeys by the injection of the filtered nasal washings from patients ill with measles. In passage experiments the disease was carried through six monkeys by injecting ground-up tissue or blood. Degkwitz⁴¹ claims to have cultured the virus of measles both in tissue cultures and in symbiosis with bacteria, although he has not produced the typical disease in monkeys or human beings with these cultures. Serum from convalescent patients causes a passive immunity in those exposed to the disease and prevents an attack if injected dur-

ing the first few days of the incubation period. The weight of evidence seems to be in favor of a filterable virus as the etiological agent of measles.

The great epidemics of measles that swept through the army camps during the World War produced pulmonary lesions that resembled closely those observed in epidemic influenza. During ordinary times among the civilian population the pulmonary lesions following measles are much less severe. Nothing comparable to the acute edematous, hemorrhagic, pulmonary consolidation found in the early stage of influenza is seen in the ordinary case. Instead, the changes in measles are similar to those found in individuals who survive the early stage of influenza and begin to show interstitial bronchopneumonia. Although large areas of hemorrhagic consolidation are not found, a halo of hemorrhage frequently encircles the yellowish peribronchial nodules.

During the past five years about 30 autopsies have been performed by one of us upon children and young adults dying of measles, and practically every specimen shows a typical interstitial bronchopneumonia. Among these cases there were several instances of lobar pneumonia and others of marked confluent lobular pneumonia. Lung abscess and fulminating streptococcus pneumonia were rare. Pleurisy and empyema, which also occurred so often as complications of both influenza and measles in the army camps during the World War, are less frequently found following measles in the civilian population during peace times.

Microscopically the interstitial infiltration is marked, and collections of large mononuclear cells are found in the alveoli more frequently than in influenza. At times these cells form multinucleated giant cells. Dense masses of fibrin are also commonly seen in alveoli surrounding thickened bronchioles, and a polymorphonuclear exudate fills the bronchioles and some connecting alveoli in areas where bronchopneumonia is present.

THE ETIOLOGY AND ANATOMICAL LESIONS OF WHOOPING COUGH

A number of different bacteria were described as causing whooping cough prior to the isolation in 1906 by Bordet and Gengou⁴² of the bacillus that is now generally accepted as the etiological agent. There are many apparently convincing reasons for regarding

the pertussis bacillus as the cause of this disease. In the first place the bacillus is associated with pertussis only, and is not found in other diseases. Bordet and Gengou first showed that this micro-organism could be isolated from the bronchial secretions during the catarrhal stage of pertussis in a high percentage of cases, and that specific, complement-fixing antibodies were present in the blood of patients convalescent from pertussis. Chievitz and Meyer⁴³ soon confirmed these observations and since then others have amply corroborated them.

These clinical observations have never received the experimental confirmation that might be anticipated in a supposedly simple bacterial disease. The experiments of Klimenko⁴⁴ on young dogs are open to question because the possibility of distemper, which is frequent in these animals, was not ruled out and it is now known that *B. bronchisepticus* has biological characteristics much like those of the pertussis organism. Other workers have not succeeded in repeating Klimenko's results. The work of Mallory, Hornor and Henderson⁴⁵ called attention to the presence of bacilli between the cilia of the bronchial epithelium in pertussis. However, various types of bacteria can be demonstrated in the same position in other human diseases as well as in spontaneous infections in dogs, rabbits and guinea pigs.⁴⁶

Some investigators have doubted the etiological rôle of the bacillus of Bordet and Gengou. Indeed a critical study of the relation of this bacillus to the disease reveals disturbing inconsistencies. The bacilli are present only in the early stages of the disease. The incubation period of pertussis is longer than that of the average bacterial disease. A vaccine prepared from the bacillus has been used for a number of years but is of doubtful value either as a prophylactic or as a curative agent. Because of this it was omitted from New and Non-Official Remedies in 1931.⁴⁷

B. influenzae has often been isolated from cases of pertussis, although Winholt⁴⁸ claims that complement fixation tests with this organism are negative in such instances. In this connection it is interesting to recall that Jochmann and Krause,⁴⁹ who were the first to describe a specific organism as the cause of pertussis, described one that later proved to be a strain of *B. influenzae*.

In 1903, and again in 1913, Manicatide⁵⁰ advanced similar claims for a bacillus he described, and reported positive complement fixa-

tion with it in whooping cough convalescents and negative reactions in normal individuals.

Oclair⁵¹ advanced the idea that pertussis is a symptom complex that may be caused by a variety of bacteria. This conclusion seems improbable however, because of the epidemiology of the disease and the development of a rather solid immunity after an attack.

Kraus⁵² suggested that the primary cause of the disease was a filterable virus and claimed good results by treating patients with sterile filtrates of pertussis sputum. Convalescent serum has recently been used as a prophylactic with suggestive results by Bradford.⁵³

Encephalitis is an infrequent complication of whooping cough, also of measles and vaccinia, although this is only remote evidence in favor of a virus since encephalitis has been known to follow other mild upper respiratory infections.

The recent discovery of intranuclear inclusions in about one-third of our autopsied cases of pertussis (McCordock⁵⁴) is strong evidence in favor of the possible rôle of a filterable virus in this disease.

The pulmonary lesions of whooping cough are much the same as those of measles. Interstitial bronchopneumonia is usually quite marked and collections of large mononuclear cells are often found in the alveoli. When present, intranuclear inclusions serve to distinguish the interstitial pneumonia of whooping cough from that of measles and influenza. These inclusions are acidophilic, staining pink with Mallory's eosin-methylene blue, and with Giemsa's stain. A clear zone separates the inclusions from the nuclear membrane to which small particles of chromatin sometimes adhere. Inclusions are found in the cells lining the alveoli, in the bronchial epithelium and occasionally in the cells of the mucous glands or ducts of the bronchial walls. In a small percentage of cases inclusions are found in liver cells about which there are foci of necrosis with infiltration of polymorphonuclear leukocytes. They are also found in similar focal necroses in the adrenal gland. The cells containing the inclusions are larger than their neighbors and have a more basophilic cytoplasm. That these inclusions bear a specific relation to pertussis is evident, since they have been found in a third of all our cases of pertussis and only twice in 100 control autopsies of children dying of various other diseases. In both of these there was also a peribronchial infiltration with large mononuclear cells and lympho-

cytes. Genuine intranuclear inclusions have undoubtedly always been present in cases of pertussis, and are not spurious or of spasmodic occurrence, or restricted to a particular locality, since we have found them in sections from autopsies performed in St. Louis during the last 15 years and have observed them in autopsy material in other distant cities as well. Feyrter⁵⁵ of Hamburg has reported intranuclear inclusions in one case of pertussis, but this is the only other instance we have been able to find in the literature in which inclusions have been associated with whooping cough.

The pulmonary complications of pertussis are similar to those of measles. Severe, confluent lobular pneumonia, and the lobar type in older children, are the only common postpertussis lesions. In our experience severe bronchiectasis is more frequently encountered in this disease than in influenza or measles. This may in part be due to the more chronic nature of pertussis and to the violent paroxysms of coughing.

DISCUSSION

The experimental work on influenza during and since the last pandemic amply illustrates the futility of attempting to explain the disease simply upon the basis of a bacterial infection. It would be unique for great epidemic outbursts of a disease like influenza to be caused by a bacterium that ordinarily is a harmless inhabitant of the nasopharynx of many healthy individuals at all times. Indeed the epidemic character of this malady resembles that of a virus disease such as measles or poliomyelitis, rather than that of a bacterial disease due to a microorganism that usually is an innocuous commensal but suddenly develops virulent characteristics.

While direct proof that influenza is caused by a filterable virus is still wanting, the character of the anatomical lesions observed in this disease furnishes indirect evidence in favor of such a view, since lesions similar to those of influenza are found in measles, a known virus disease, and can be produced experimentally in animals with vaccine virus. In accordance with this interpretation the influenza virus is probably pneumotropic and produces in the vast majority of individuals affected a severe, although self-limited, respiratory disease. Some patients succumb a few days after the onset of symptoms and the lungs of these individuals show a hemorrhagic and edematous lobular consolidation, a patchy destruction and desqua-

mation of alveolar and bronchial epithelium, and, at times, foci of necrosis in which all the pulmonary structures are killed *en masse*. This corresponds to Wolbach's first stage, or to the acute diffuse fulminating influenzal pneumonia of Winternitz. We have shown that lesions identical with these can be produced with vaccine virus. By itself this fact is of no significance, since many injurious substances can produce the same damage, as Winternitz has shown in experiments with war gases. The importance of vaccine virus in this connection, however, becomes at once apparent when it is recalled that if allowed to act in a more dilute form for a longer time the same virus incites the interstitial changes found in the later stages of influenza, and when it is used in combination with bacteria most of the complications of this disease can be reproduced in animals. This is far more than can be accomplished with either the war gases or with the influenza bacillus, no matter how much its virulence has been increased, in spite of the capacity of each of these agents to produce severe initial damage.

In the early stage of the disease the lungs often show in addition a bronchiolitis, and any bacteria present are found in the exudate within the bronchioles. However, at this time bacteria are scarce, as pointed out by Goodpasture, and consist principally of influenza bacilli and occasionally streptococci.

The majority of patients, in whom the disease is not of short duration, survive this initial period, subsequently developing more chronic virus lesions and one or more of a variety of pulmonary complications due to an assortment of secondary bacterial invaders. Interstitial bronchopneumonia is the most constant additional lesion in individuals who survive the first week. Three distinct elements can be recognized in the lesion that MacCallum has designated interstitial bronchopneumonia. Two of these, namely, the cellular infiltration of the interstitial tissue and the non-purulent peribronchial alveolar exudate composed of large mononuclear cells, dense compact masses of fibrin or red cells, we regard as a histological manifestation of the action of a virus upon the lung and include it in the description of pure virus pneumonia. Both of these changes can be produced experimentally with virus alone and they are also found in individuals dying of a virus disease, such as measles, in regions of the lung where bacteria cannot be demonstrated. The third element, the lobular or bronchopneumonia, is the

result of secondary bacterial infection that calls forth a polymorphonuclear cell exudate in the bronchioles and in groups of connecting alveoli.

The *Streptococcus hemolyticus*, *B. influenzae*, and other bacteria have been regarded as the cause of interstitial bronchopneumonia by different authors. Such a view seems unlikely, since the interstitial part of the lesion may occur in the absence of bacteria, and many examples of pulmonary infection with these bacteria exist without a suggestion of the interstitial infiltration. Again, many cases of measles and whooping cough present a well developed interstitial infiltration, and neither the influenza bacillus, the streptococcus, nor any other bacterium is constantly associated with this lesion.

The influenza bacillus has been found more frequently in each of these diseases associated with interstitial bronchopneumonia than any other organism. It will be recalled that Blake found it present in 80 per cent of the cases of measles during the influenza epidemic, and others have reported its presence in the same disease during interepidemic periods. Odaira,⁵¹ Winholt,⁴⁸ Kristensen⁵⁶ and others have isolated the bacillus from cases of whooping cough. Little significance, however, can be attached to the presence of *B. influenzae* in these three diseases when its frequency in other conditions is considered. Working in Chicago over a 3 year period Bourn⁵⁷ showed that influenza bacilli could be demonstrated in about 50 per cent of individuals suffering from pneumonia of all types, in 40 per cent of patients with pulmonary tuberculosis, in 30 per cent with various other respiratory disorders, as well as in about 20 per cent of the healthy population. If *B. influenzae* were the cause of interstitial bronchopneumonia is it not remarkable that it should produce this lesion in three diseases only — influenza, measles and whooping cough — when it also is present in such a high percentage of other respiratory diseases? True interstitial bronchopneumonia has not been reproduced in animals by the injection of the influenza bacillus or other associated respiratory bacteria.

Some children dying of bronchial diphtheria show a cellular infiltration of the walls of affected bronchi, in addition to a lobular pneumonia. This reaction has occasionally been cited as an instance of interstitial bronchopneumonia caused by a bacterium. However, the cases of diphtheria we have studied can be distinguished easily

from those of measles or whooping cough. In diphtheria the infiltrating cells are practically all lymphocytes and plasma cells with polymorphonuclear leukocytes near the epithelial surface, while the large mononuclear wandering cells found so commonly in interstitial pneumonia are scarce. The groups of alveoli encircling infiltrated bronchi do not, as a rule, contain the dense compact masses of fibrin or the collections of large mononuclear cells, as do those in measles and whooping cough. Instead they are uniformly filled with the acute inflammatory exudate characteristic of lobular pneumonia. The diffusion of toxin into the bronchial wall from diphtheria bacilli growing upon the epithelial surface seems an adequate explanation of the cellular infiltration in this disease.

There is other indirect evidence substantiating the theory that the primary cause of interstitial bronchopneumonia is a virus. Recently we examined the lungs of a child who died of generalized vaccinia and found a typical interstitial bronchopneumonia, as well as abscesses which were due to a secondary staphylococcus infection. Through the courtesy of Dr. T. M. Rivers, sections of two cases of human psittacosis and one of an experimental infection in a monkey have been obtained. These also show the interstitial reaction and in the sections of one element of the more acute hemorrhagic virus pneumonia can be made out. Several examples of interstitial bronchopneumonia in dogs, which showed respiratory complications during a spontaneous distemper infection, have been found in Roman's ⁵⁸ material. These three diseases, as well as measles, are caused by viruses, and associated with each examples of interstitial bronchopneumonia can be found. The discovery of intranuclear inclusions in pertussis also points to the association of a virus with interstitial bronchopneumonia in whooping cough.

Specific bacteria have been in the past, or are at present, regarded by some workers as the etiological agents of influenza, measles and pertussis. If viruses are held to be the primary cause of these diseases the associated bacteria naturally will be regarded as secondary invaders. In the case of measles the majority of workers already accept such an interpretation. The studies initiated by the last pandemic have cast such grave doubt upon the causal relation of *B. influenzae* to epidemic influenza in man that few investigators still regard it as the cause of this disease. The pertussis bacillus, on the other hand, is almost universally regarded as the cause of whoop-

ing cough. The evidence in favor of this conclusion seems unassailable, and bacteriologists will point to the high incidence of the bacillus in this disease, its absence in other conditions and the presence of specific antibodies in the serum of convalescents. Indeed the bacillus may still have a very direct association with the disease, and future work alone will determine this relation. In the meantime, however, in connection with both influenza and pertussis, it is interesting to recall the history of three other diseases, namely, hog cholera, canine distemper and psittacosis, which for years were regarded as being caused by specific bacteria but now are known to be primarily due to viruses. If the virus nature of influenza and pertussis can eventually be proved by direct experiments, hog cholera and dog distemper will furnish perfect analogies of a similar revision in our conception of the etiology of a disease.

In 1886 Salmon and Smith⁵⁹ described *B. suispestifer* as the etiological agent of hog cholera. This organism was found almost constantly associated with the spontaneous disease and produced in experimental animals a condition which closely resembled the natural infection, even to the extent of showing similar pathological lesions. This work apparently so firmly established the etiological rôle of *B. suispestifer* that for 17 years it was universally regarded as the cause of hog cholera, just as the pertussis bacillus is today so universally thought to be the primary cause of whooping cough. At length, de Schweinitz and Dorset⁶⁰ in 1903 proved that the disease could be transmitted with bacteria-free filtrates of organs and blood from infected animals and that *B. suispestifer* was a common secondary invader.

The history of distemper repeats the same sequence of events. In this disease, as in pertussis, the almost constant presence of specific antibodies in the blood of convalescents seemed to establish beyond question the etiological rôle of the associated organism *B. bronchisepticus*. A vaccine was prepared and for a time enthusiastically used, but, like the pertussis vaccine, it eventually proved to be of doubtful value and was discarded. Finally, Roman⁵⁸ in 1925, and Dunkin and Laidlow⁶¹ in 1926, proved that the disease was caused by a filterable virus.

In a study of swine influenza Shope⁶² has shown that this disease is caused by a filterable virus and *H. influenzae suis* acting together. The injection of cultures of the bacillus alone is without

effect, and animals receiving only the filterable virus develop a mild "filtrate disease" which is not typical swine influenza. The pathological lesions of swine influenza⁶³ resemble those of human epidemic influenza. This work supplies additional experimental evidence in support of our conclusion that the pulmonary lesions of epidemic influenza, measles, and pertussis are in all probability due to the combined action of a filterable virus and bacteria.

Interstitial bronchopneumonia is regarded by us as the type reaction to the presence of a virus and a bacterium, but in no sense specific for a certain virus or a particular microorganism. Such a distinct lesion is usually type specific, and is caused by a particular stimulus or by several related stimuli, especially when the lesion is restricted to a group of related diseases. An acute inflammatory exudate is an example of a specific reaction that is called into play in the presence of pyogenic bacteria, and as such it is a useful index of infection even though sterile necrotic tissue or chemical irritants can induce the same reaction. A tubercle is the type reaction to the presence of foreign bodies in the tissue and although associated with a large variety of foreign bodies, tubercles, nevertheless, are helpful in the diagnosis of tuberculosis. However, tuberculosis can exist apart from tubercles or giant cells, as for instance in tuberculous meningitis or pneumonia, in which a large number of tubercle bacilli excite an exudative reaction which is essentially an acute inflammation. Similarly, we have shown that vaccine virus can produce two distinct types of reaction in the rabbit's lung. One has been designated as hemorrhagic virus pneumonia because it consists essentially of hemorrhage, edema and necrosis, and the other, interstitial virus pneumonia because of the marked accumulation of wandering cells in the interstitial tissue. The latter type of reaction is one of the histological components of interstitial bronchopneumonia in man and represents, we believe, the action of some virus upon the lung tissue. The lobular, or bronchopneumonic constituent of the lesion is the result of one or more of a variety of pyogenic bacteria entering the lung as secondary invaders after the virus has impaired the defense mechanisms that ordinarily prevent these microorganisms from infecting the lung. In this sense we regard interstitial bronchopneumonia as a type specific reaction due to the combined presence of a virus and bacteria.

SUMMARY AND CONCLUSIONS

Vaccine virus injected into the lungs of rabbits can incite two different types of reaction depending upon its concentration. A strong virus tends to produce a hemorrhagic, edematous consolidation and irregular areas of necrosis with hemorrhage that resemble infarcts. Polymorphonuclear leukocytes infiltrate the necrotic tissue if the animal survives. This acute reaction has been termed hemorrhagic virus pneumonia. On the other hand, a dilute virus calls forth a proliferative cellular lesion, which we have called interstitial virus pneumonia, in which the walls of the bronchi, the alveoli and the blood vessels become thickened due to an infiltration that consists principally of mononuclear cells.

The lesions of hemorrhagic virus pneumonia in rabbits are similar to the hemorrhagic and edematous lobular consolidation with foci of necrosis found in the lungs of individuals dying a few days after the onset of symptoms of epidemic influenza. The cellular infiltration of interstitial virus pneumonia in rabbits resembles the interstitial accumulation of cells seen in the interstitial bronchopneumonia that so frequently accompanies influenza, measles and whooping cough in man. It is impossible to reproduce the complete picture of interstitial bronchopneumonia with vaccine virus alone, for the lobular or bronchopneumonic component is always lacking, but it can be superimposed upon interstitial virus pneumonia by injecting bacteria into the lungs of a rabbit subsequent to the introduction of dilute vaccine virus. Not only can the complete picture of interstitial bronchopneumonia be reproduced by the combined use of vaccine virus and bacteria, but also practically all the pulmonary complications of influenza, measles and whooping cough, with the exception of lobar pneumonia.

Interstitial bronchopneumonia can be reproduced by vaccine virus and bacteria, and it also is found in known virus diseases such as measles, generalized vaccinia, psittacosis, and in some cases of spontaneous distemper in dogs that develop respiratory complications. Interstitial bronchopneumonia is therefore regarded as the type reaction for the combination of a virus and bacteria, although in no sense specific for a particular virus or bacterium.

Influenza and pertussis are the two human diseases associated with interstitial bronchopneumonia for which satisfactory proof of

the presence of a virus is lacking, although the type of the anatomical lesions associated with each is indirect evidence of the action of such an agent. In the case of pertussis the recent discovery of intranuclear inclusions in about one-third of our autopsied cases, and their absence in control material, support the idea that a virus is associated with interstitial bronchopneumonia and indicate that a filterable virus may also play a rôle in the cause of this disease.

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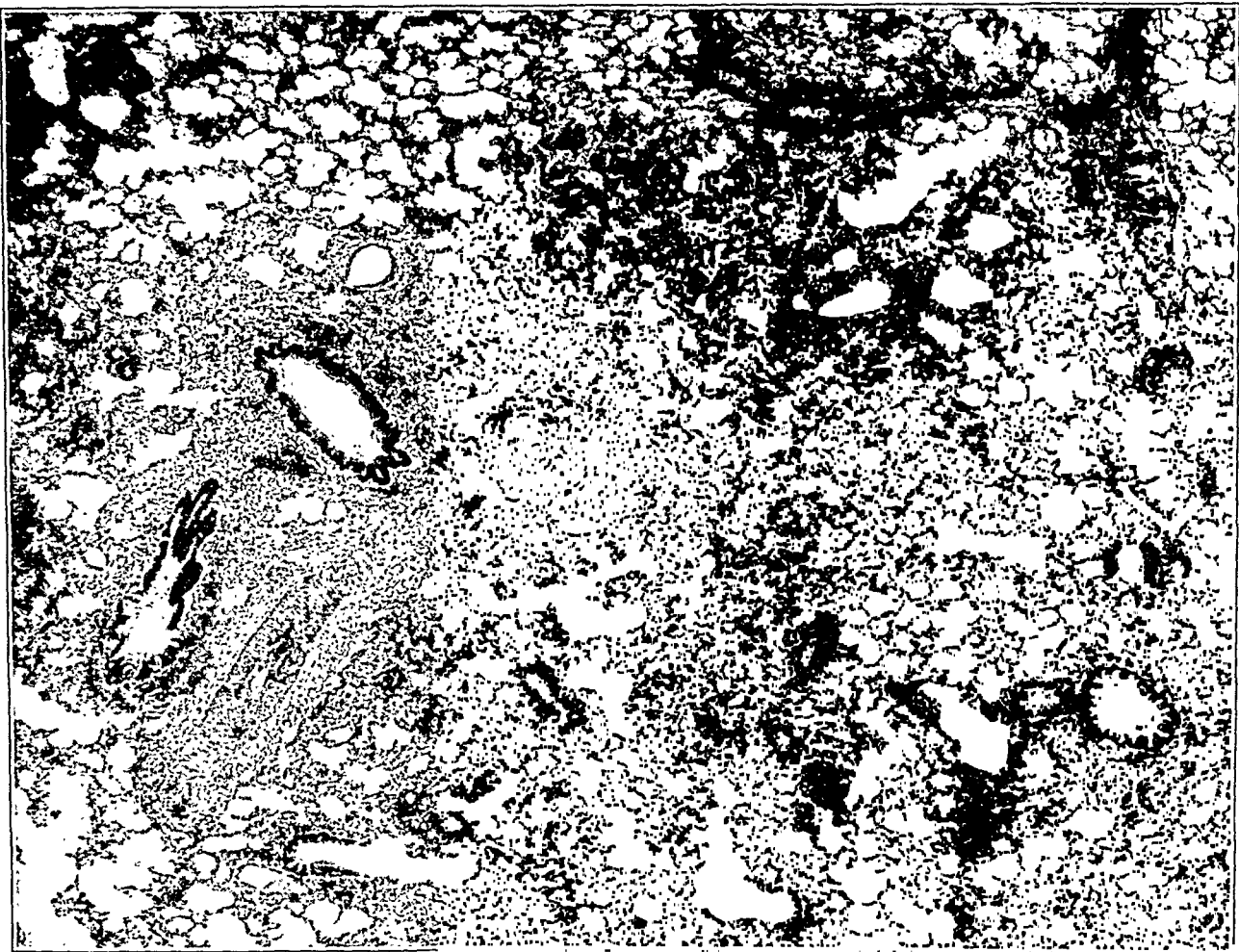
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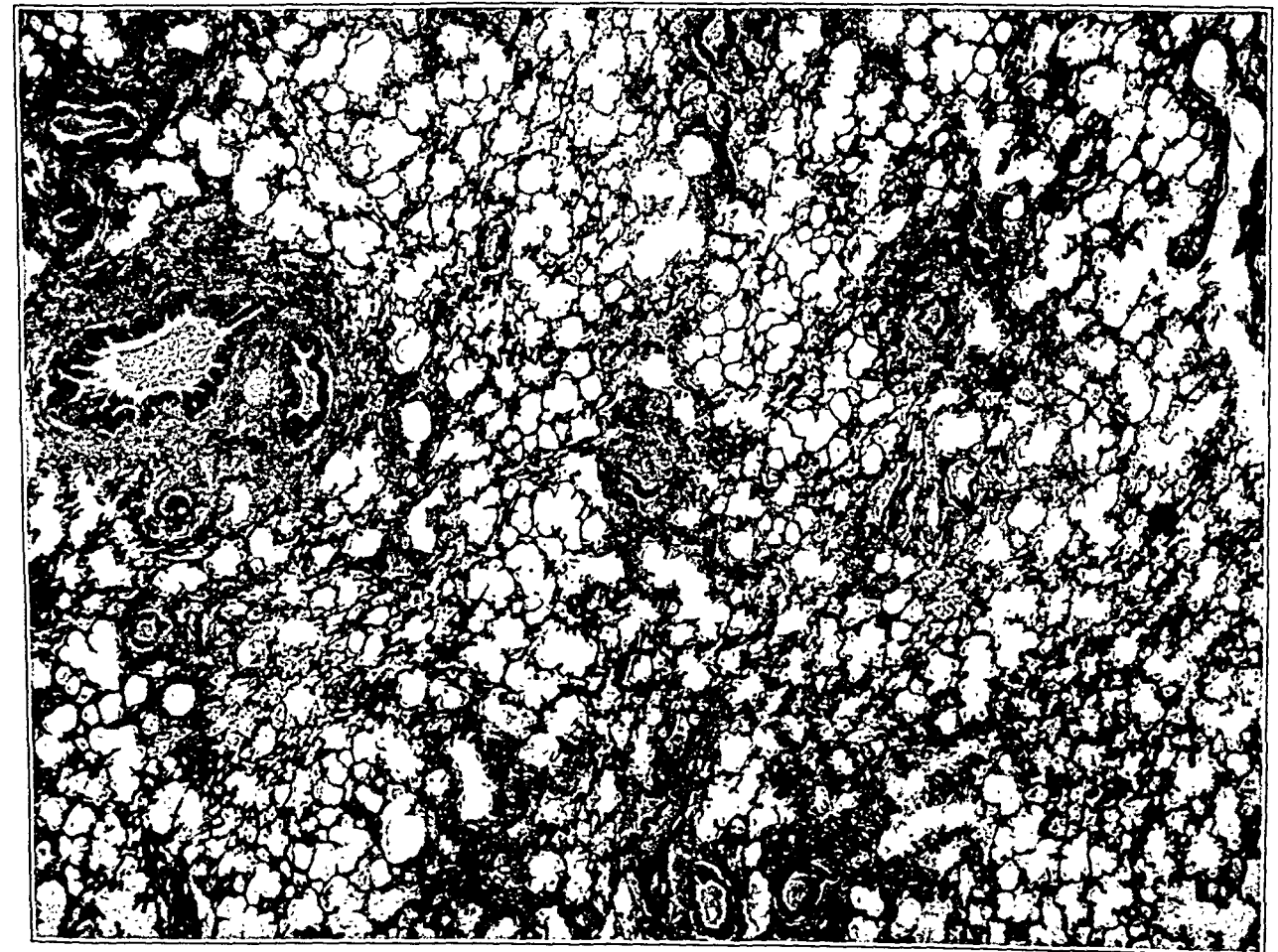
DESCRIPTION OF PLATES

PLATE 32

- FIG. 1. Interstitial virus pneumonia in a rabbit 5 days after inoculation of dilute vaccine virus. A zone of infiltration encircles each bronchus and many of the alveolar walls are thickened. No bacteria could be demonstrated in this lung. $\times 30$.
- FIG. 2. Interstitial bronchopneumonia following measles in a child. Note the peribronchial infiltration and the thickening of many of the alveolar walls. Most of the bronchi contain an exudate composed of polymorphonuclear leukocytes, fibrin and bacteria, which is not present in the animal lung illustrated above. $\times 30$.



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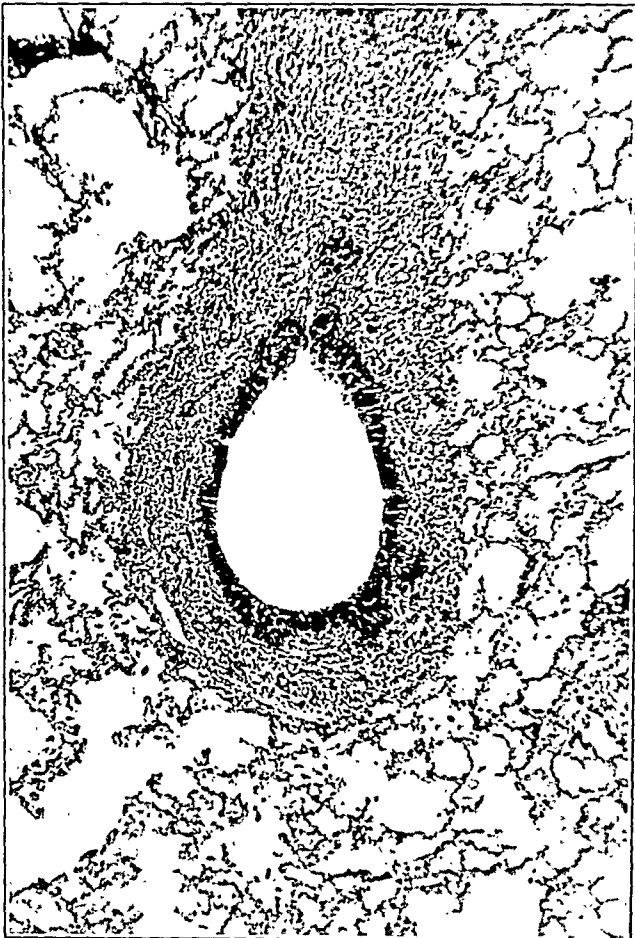
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PLATE 33

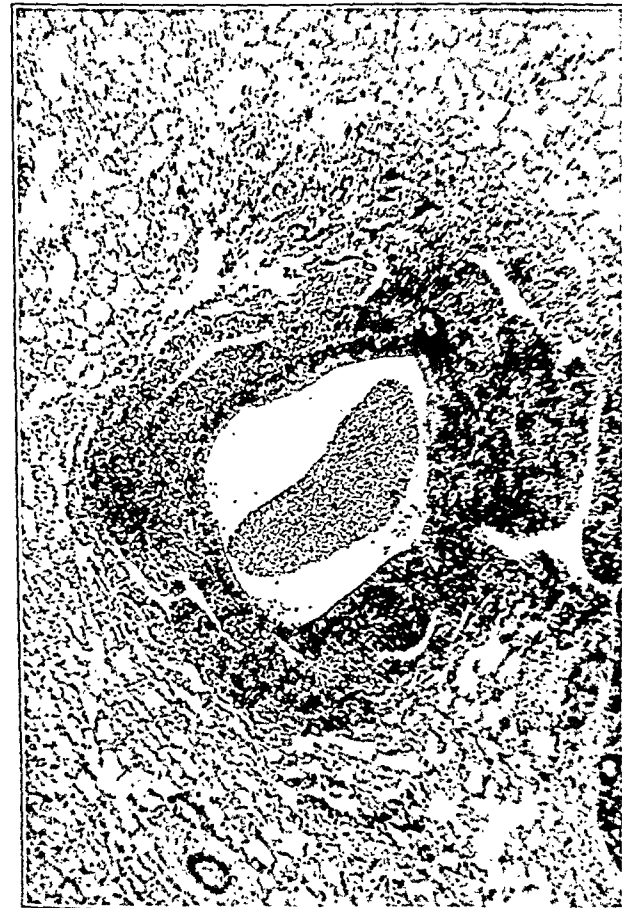
- FIG. 3. Bronchiole of animal lung 7 days after injection of virus. The peribronchial tissue is densely infiltrated with mononuclear cells. The lumen is clear. $\times 80$.
- FIG. 4. Similar peribronchiolar infiltration in the lung of a child who died of pertussis. No bacteria were demonstrated in sections from this region of the lung. $\times 80$.
- FIG. 5. Peribronchial infiltration and polymorphonuclear exudate in the lumen of the bronchiole. Lung of animal after the injection of virus and bacteria. $\times 80$.
- FIG. 6. Bronchiole from the lung of a child who died of pertussis showing lesions similar to those illustrated in Fig. 7. Bacterial stains of adjacent sections contain Gram-negative bacilli and Gram-positive cocci. Cultures of this lung revealed *B. influenzae* and a hemolytic staphylococcus. $\times 80$.



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4



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PLATE 34

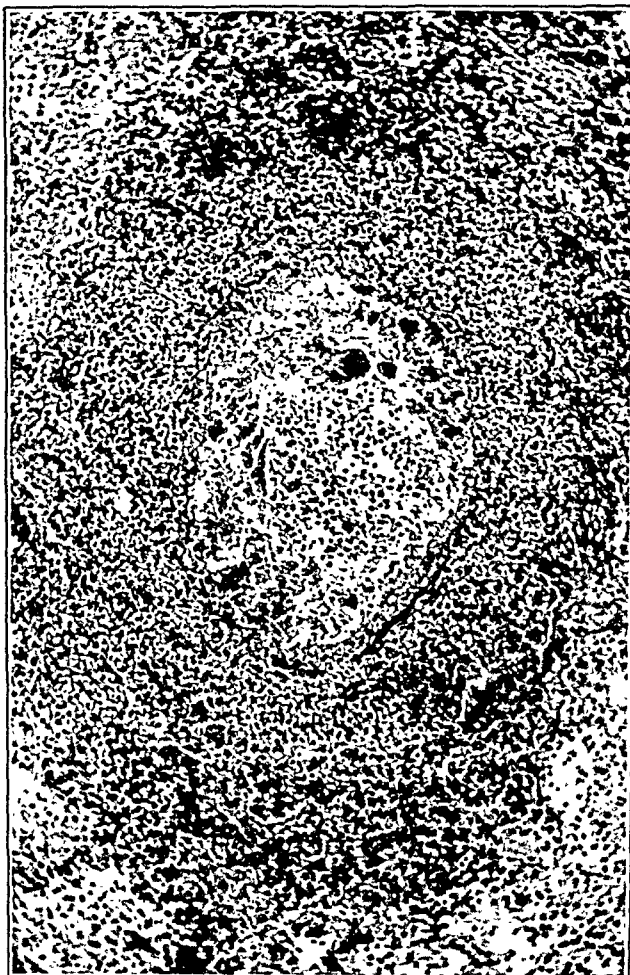
FIG. 7. Necrosis of the wall of a bronchiole in an animal after the injection of vaccine virus and bacteria. $\times 100$.

FIG. 8. Necrosis of the wall of a small bronchus from a case of epidemic influenza. $\times 100$.

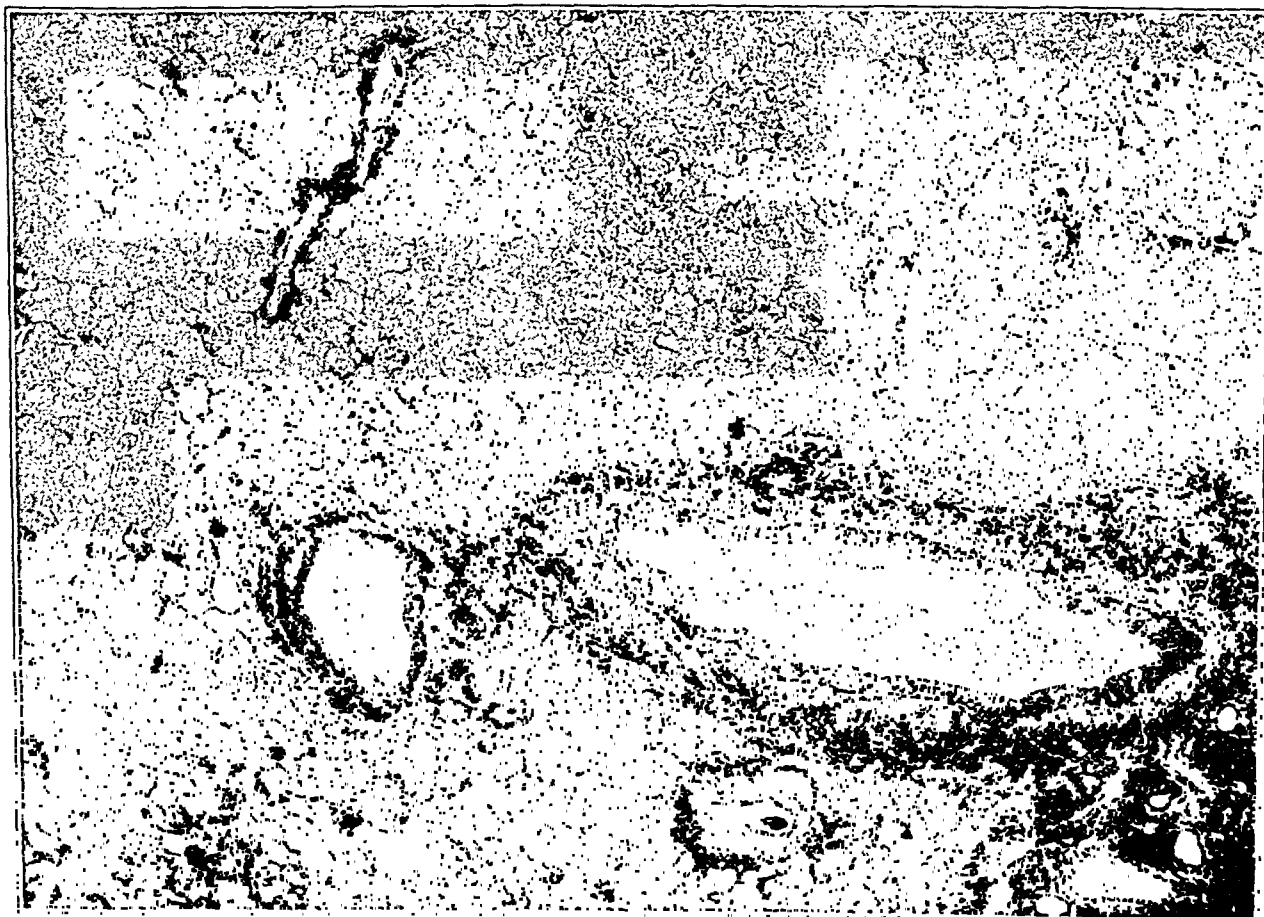
FIG. 9. Experimental interstitial bronchopneumonia in an animal after the injection of virus and bacteria. There is a dense collection of mononuclear cells about the two bronchi shown in the lower part of the print. The lumen of each bronchus contains an exudate composed of polymorphonuclear cells, fibrin and bacteria. The lungs of this animal showed a lobular pneumonia that in places was confluent, as in the portion illustrated, in which all the alveoli are filled with a typical polymorphonuclear exudate. $\times 25$.



7



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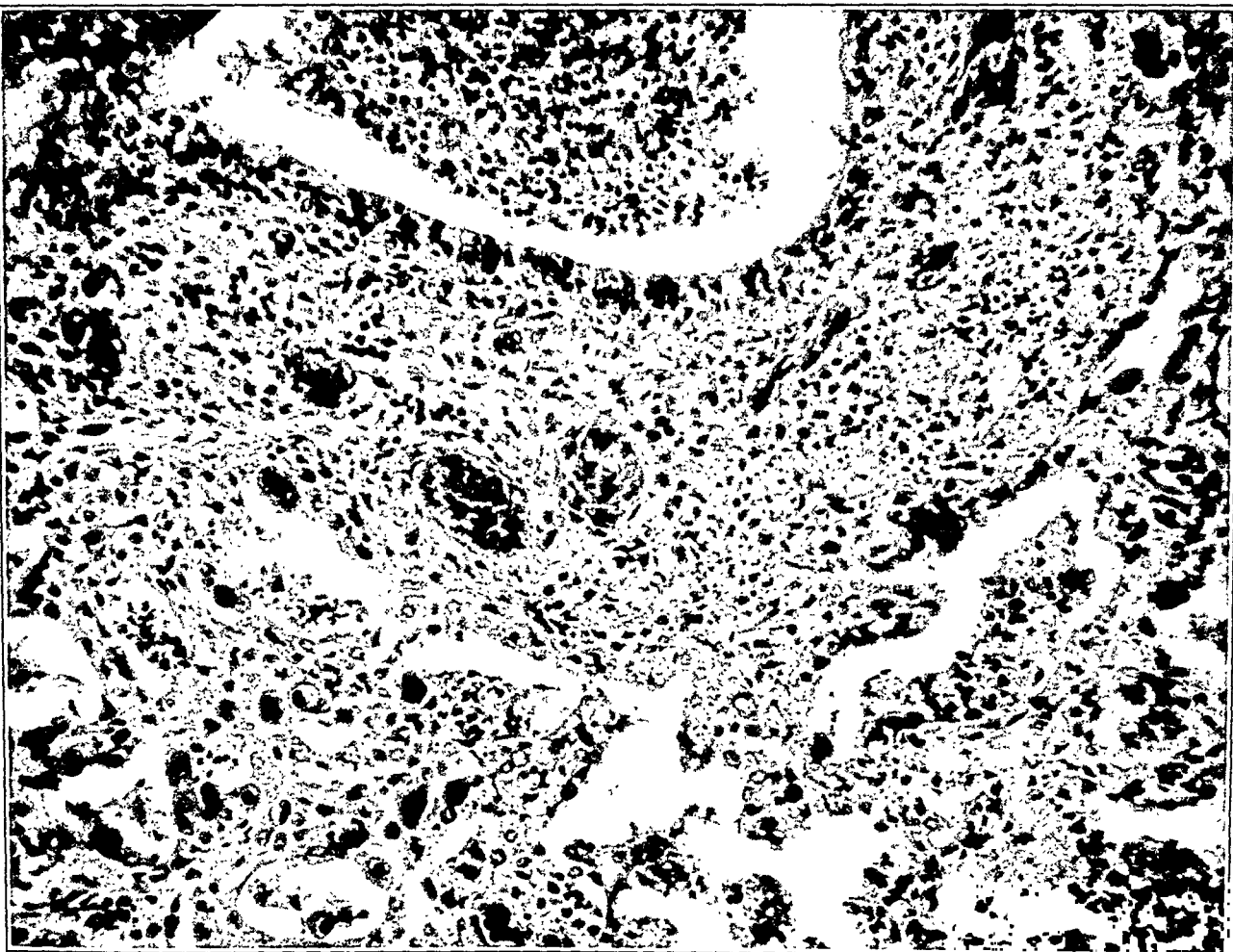


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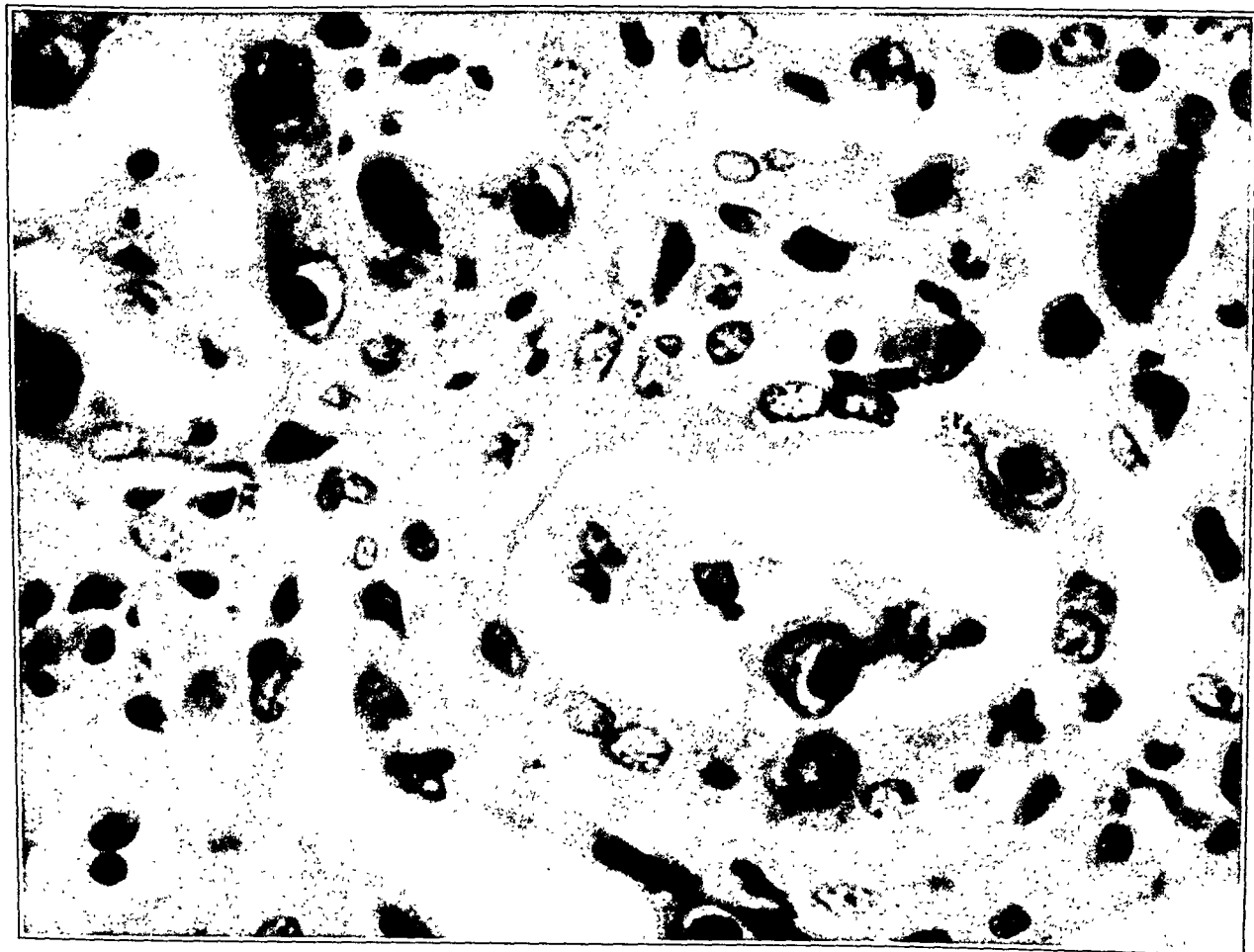
PLATE 35

FIG. 10. Interstitial bronchopneumonia in the lung of a child who died of pertussis about 3 weeks after the onset of symptoms. The peribronchial and alveolar wall thickening and the cellular infiltration are conspicuous. A leukocytic exudate is present in the lumen of the bronchiole. In the lower right-hand portion of the field intranuclear inclusions can be seen in the cells lining the alveoli. A portion of this region is shown in a higher magnification in Fig. 11. $\times 300$.

FIG. 11. The intranuclear inclusions of pertussis. The alveolus in the lower half of the field and to the left contains a detached cell with an inclusion, but elsewhere the inclusions occur in cells lining the alveoli. $\times 850$.



I0



II

MICROTECHNICAL DEMONSTRATION OF INSOLUBLE LIME SALTS IN TISSUES *

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With microtechnical methods hitherto described insoluble lime salts can be demonstrated only if the tissue to be examined is soft enough for the microtome knife, *i.e.*, if it contains only small quantities of finely dispersed calcareous material. Under such conditions the Kóssa¹ silver impregnation or the Roehl lead sulphide reaction yields very instructive pictures. If, however, the tissue can be made sectionable only by decalcification, lime salts no longer can be demonstrated as they have been removed by the decalcifying agent. There are some methods for staining tissues which have contained calcium salts, *e.g.*, the simple hematoxylin stain, which imparts a characteristic dull dark blue color to decalcified tissues, or the Antonow² safranin-light green stain, and several others. They allow a fair orientation as to distribution of lime salts in the tissues, but they are, however, entirely unsuitable for more exact morphological research. It has been recommended, also, to make sections of hard tissue without decalcification, as even chips obtained in this way can be used for the Kóssa or Roehl method. By this method, however, as Antonow strikingly remarks, lime salts are liable to be demonstrated more readily by notches of the microtome knife than by staining.

With the method to be described insoluble lime salts of tissues can be demonstrated even in the hardest tissues in a very clear and selective manner, and in this way a relatively unknown field of normal and pathological histology is opened for scientific research.

I should like to describe the method more fully than is customary with new microtechnical methods, as it belongs to that small group of microtechnics which have a firm chemical basis.

It has the following basic principle: the lime salts must be stained or impregnated in blocks of fixed tissue before decalcification and

* Read before the First Conference of the Association of Hungarian Pathologists, Budapest, June 6th and 7th, 1932.

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sectioning. Once the lime salts are stained, the tissues can be decalcified and embedded by routine methods. The stain remains at the site of the lime salts and if the reaction used is selective and to a certain extent quantitative, equivalent pictures must result, in the sense of Nissl.

Silver salts seemed to be quite suitable for the purpose mentioned. The lime salt content of bones and other calcareous deposits consists for the greater part of calcium phosphate and, to a lesser degree, of calcium carbonate. As silver phosphate is much less soluble than calcium phosphate, and silver carbonate also considerably less soluble than calcium carbonate, it is to be expected that if tissue blocks containing lime salts are put into a solution of silver nitrate a reaction will take place between the calcium and silver salts, with the result that calcium will be supplanted by silver, at least in the phosphate part, in an inverse proportion to the solubility of calcium and silver phosphate, respectively. This supposition has been verified by the experiments of P. Spanyol, chemical engineer. He poured an excess of a 0.1 normal silver nitrate solution upon accurately weighed quantities of calcium phosphoricum dibasicum "Kahlbaum" (a German brand of chemically pure dibasic calcium phosphate). The white powder at once became lemon yellow. After two days the silver nitrate solution was titrated and it could be stated that about 96 per cent of Ca had become supplanted by Ag.

The silver phosphate thus obtained was then reduced to metallic silver. Then the lime salts that did not partake in the reaction were removed by decalcification. The blocks thus treated were embedded and cut. In the sections black metallic silver was seen at the site of the lime salts.

This was the chemical basis on which I developed a practical procedure. Several hundred blocks of tissue containing insoluble lime salts (bones, sclerotic vessels, calcified tumors, tuberculomata) have been utilized. I shall first describe the method in detail and then describe the results.

TECHNICAL METHOD

1. *Cutting of Fresh Tissues for Fixation:* Blocks of tissue should not be thicker than 1 or 2 mm. The thinner the block, the better the result. Thicker blocks will remain unstained in their central parts.

2. *Fixation*: Formaldehyde seemed *a priori* to be unsuitable, as it itself reduces silver salts and in this way the blocks are prone to contain precipitated silver granules. On the other hand it always contains traces of formic acid, by which lime salts are easily dissolved. In general, no acid fixing fluids should be used because of the effect just mentioned. Salts of heavy metals are unsuitable because their phosphates likewise are insoluble. All bichromates and chlorides must be avoided too, in view of the later silver impregnation. There remain only three fixing methods: alcohol, acetone, and boiling. All three methods yield satisfactory results. I prefer, however, alcohol. Blocks should be kept for 2 to 4 days in 80 to 96 per cent ethyl or methyl alcohol. All dilution should be made with distilled water. Because of its strongly shrinking and hardening properties acetone is better not used. For boiling, a 2 per cent potassium nitrate or a 5 per cent alum solution in 20 per cent alcohol can be used. Aqueous solutions of these salts are not suitable for this method, as in distilled water calcium phosphate is markedly soluble (11 mg. per 100 cc.), whereas its solubility in 20 per cent alcohol is negligible (less than 1 mg. per 100 cc. (Spanyár)). If boiling is preferred, the blocks are dropped into the boiling solution and kept in it for 5 minutes.

After fixation blocks are washed in distilled water for 3 to 4 hours.

3. *Impregnation*: A 1.5 per cent solution of silver nitrate is used. Blocks are kept in it for 6 to 10 days at room temperature. Stronger solutions act more quickly, but they are prone to darken the blocks. Higher temperatures have the same effect. A gauze pad is put under the blocks in order to facilitate penetration by the solution. The silver solution is to be changed once or twice. The harder the block on account of its lime salt content, the longer the time required for impregnation. In the silver solution the parts containing lime salts become lemon yellow within a few minutes (silver phosphate). This color changes in several days into a pale brownish yellow. The tissues ought not to become darker, particularly not in their calcium-free parts. If, nevertheless, they should get a darker brown color, which almost never happens within 14 days, impregnation must be stopped.

4. *Washing of Tissues*: Now the blocks are thoroughly washed in large quantities of distilled water, changed 4 to 5 times daily for 3 to 4 days, until the last portion of decanted washing water does not show the slightest turbidity when mixed with hydrochloric acid. This

is very essential as blocks insufficiently washed are liable to become filled with a black-brown, granular precipitate.

5. *Reduction*: The following substances have been tried: photographic developers (such as metol, hydroquinone, pyrogalllic acid, amidol), rongalite, neoarsphenamine, dextrose, formaldehyde, sodium nitrate, sodium sulphite and sodium hypophosphite. Of these, the photographic developers, rongalite and neoarsphenamine are too violent in their action. They reduce not only the inorganic silver salts but also the albuminates. The blocks become quite dark and the sections thus obtained are very dirty, loaded with an amorphous black precipitate. Dextrose, even in an alkaline medium, has a too weak reducing power. Formaldehyde has the drawbacks both of the photographic developers and of dextrose, as it is too weak and not selective in its action. Sodium nitrate does not reduce at all. Sodium sulphite yields very selective, sharp pictures, but the blocks are liable to contain a heavy precipitate. This is explained by the fact that many insoluble silver salts are dissolved in an excess of sodium sulphite, a complex sodium silver sulphite anion being formed. The latter is rather unstable and very soon metallic silver is precipitated from the solution. A most ideal reducer seems to be sodium hypophosphite. It reduces with an almost absolute selectivity only inorganic silver phosphate and carbonate, whereas it does not act upon albuminates. This is all the more interesting as it is difficult to obtain reduced silver phosphate by hypophosphites in the test tube when using pure chemicals. If a hypophosphite solution is poured over silver phosphate precipitated upon chemically pure calcium phosphate no changes will be observed for days; darkening begins much later and progresses but slowly. It has been noticed by Kóssa that silver phosphate formed from the calcium phosphate of tissues shows a different behavior from that formed from chemically pure calcium phosphate, the former being incomparably more light-sensitive than the latter. He assumes the possibility that the reaction described by him is due not to phosphates, but to an albuminous contamination. It is not easy to solve this problem. In any case the test tube experiment described above cannot be materially hastened by admixture of serum (author's own experiment). It seems to be most probable that a difference of dispersion plays an important rôle. Light sensitiveness of silver bromide emulsions likewise depends to a high degree on the amount of dispersion.

Sodium hypophosphite is used in a 5 per cent aqueous solution. The solution should be prepared fresh and made slightly alkaline by adding 4 to 5 drops of 0.1 normal sodium hydroxide solution to each 100 cc. of the fluid to counteract its acidity, as silver phosphate is markedly dissolved even by weak acids. Selective blackening of the calcareous parts can be noticed after about 30 minutes and reduction is completed within 4 to 8 days, depending on the thickness of the blocks. Sometimes the hypophosphite solution becomes brown. In this case it should be replaced by a fresh solution. It may not show, however, even the slightest turbidity, as this indicates insufficient washing of the blocks after impregnation. The result will be a blurring precipitation. The blocks themselves, except the calcareous parts which become black, do not change their color during reduction, or at best they become pale brownish.

6. After reduction the blocks are washed once more in running water for 3 to 4 hours.

7. Fixation for 2 days in a 3 to 5 per cent solution of sodium thio-sulphate. The solution should be changed once or twice. All procedures mentioned hitherto beginning with impregnation should be performed in the dark. After fixation, however, the blocks have become insensitive to light and they can be treated further in daylight.

8. Washing in running water for at least 24 hours.

9. *Decalcification*: For this purpose acids which attack metallic silver (nitric acid, and to a lesser degree hydrochloric acid) must be discarded. The best is a 6 to 8 per cent solution of sulphosalicylic acid, which, upon my suggestion, has been used as a decalcifying agent for a number of years at the First Institute for Pathological Anatomy of the University of Budapest, giving great satisfaction. It does not attack metallic silver, nor is nuclear staining materially impaired by it. Decalcification is accomplished within 1 to 3 days, according to the thickness or the hardness of the blocks. It is advisable to change the acid solution once or twice. After decalcification blocks should be washed for the last time thoroughly.

This is the procedure proper. The blocks are now embedded by one of the routine methods. They are easily cut and can be stained by most staining methods.

DISCUSSION

In the finished sections lime salts appear with a striking clearness a deep black on an entirely unstained background. Sometimes the most bizarre formations are observed such as (*a*) parallel arranged bulky disks lying at right angles to the connective tissue strands so as to form rows, connected by very fine tendril or branch-like filaments, (*b*) often spider or milliped-like figures, and (*c*) sometimes nothing but amorphous granules (see illustrations). Here I should like to mention that according to Schmorl³ the Kóssa silver stain is unsuitable for study of the finer structures, as silver phosphate is a crystalline precipitate. I find that this objection is not justified. The fine tendril-like formations speak against composition of coarser crystals. Moreover, we have the Golgi impregnation which is based on the formation of a silver bichromate precipitate. If one mixes a drop of potassium bichromate and a drop of silver nitrate on a glass slide, one can observe under the microscope how coarse the crystals thus formed are. And yet nobody would condemn as being unsuitable for finer microscopic work the Golgi impregnation method which yields the most admirable results in neurohistology.

I am obliged to mention a shortcoming of my method and this is the relatively slight penetrating power of the impregnation. It often happens even after 14 days impregnation that only a more or less broad marginal zone of the thicker calcareous masses becomes impregnated, whereas the center remains unstained. This phenomenon has probably its underlying cause in the extremely slight permeability of the silver phosphate membrane which is formed upon the surface of the calcareous parts by the silver nitrate solution. A somewhat similar phenomenon is observed with bones fixed in alcohol when stained with hematoxylin: the characteristic dull dark blue color is often shown only by a thin marginal zone. Schmorl is disposed to explain this fact by chemical factors. He admits, however, that this explanation is not entirely satisfactory. The following facts furnish evidence that it is due entirely to the unsatisfactory permeation by the silver nitrate solution: (*a*) the longer the impregnation, the broader the stained zone; (*b*) that there is no question of unsatisfactory permeation by the reducing agent, which could be very well possible on account of the probably extremely slight permeability of the metallic silver membrane formed, has been demon-

strated by the following experiment. A great number of blocks have been impregnated for 3 to 14 days. One-half of these have been reduced by the method described. The other half have been directly decalcified with hydrochloric acid after thorough washing. The embedded blocks have been cut and the sections reduced in a hydroquinone solution. No other difference could be observed between sections prepared in these two different ways except that those decalcified with hydrochloric acid were exceedingly dirty and crowded with an amorphous black precipitate. The breadth of the stained zones, however, was exactly the same.

I think, however, that the shortcoming described cannot be considered as a grave defect. The pathologist is generally much more interested in the peripheral zone of the calcified foci, where calcification or halisteresis is active, than in the central parts where he can see for the most part only dead, inert material. On the other hand, at the boundary of the unstained, deeper layers the silver deposit is so densely black and so opaque that no structure whatever can be observed there, even in the case of a perfect permeation.

Attention is called to the fact that this method is suitable for demonstration of lime salts also in gross specimens, osteoplastic tumors and so on. Slices cut or sawed out from the material are fixed in alcohol, then kept for several hours in a silver nitrate solution until an intense lemon yellow color appears throughout the calcareous parts. After this the slices are thoroughly rinsed in distilled water and reduced in the hypophosphite solution for 24 hours. After fixing them in a thiosulphate solution and washing once more, they can be preserved in the fluids generally used for this purpose. The calcareous parts with their blackness on a background of natural colors give a very clear, striking picture.

Finally I wish to remark that preparations, microscopic as well as macroscopic, made according to my method are very durable. They withstand even direct sunshine for a long time.

SUMMARY OF METHOD

1. Cut or saw thin blocks of fresh tissue 1 to 2 mm. thick.
2. Fix in 80 to 96 per cent alcohol, or boil in one of the solutions mentioned. After this wash blocks in distilled water for 3 to 4 hours.
3. Impregnate in 1.5 per cent silver nitrate solution for 6 to 10 days. Change silver solution once or twice.

4. Wash for 3 to 4 days in distilled water changed daily 4 to 5 times, until the last washing water decanted does not show the slightest turbidity when mixed with hydrochloric acid.

5. Reduce in a 5 per cent solution of sodium hypophosphite. Before use add 4 to 5 drops of a 0.1 normal sodium hydroxide solution to each 100 cc. of reducer. Keep blocks in reducer for 4 to 8 days.

6. Wash in running water for 3 to 4 hours.

7. Fix in a 3 to 5 per cent solution of sodium thiosulphate for 2 days.

8. Wash in running water for at least 24 hours.

9. Decalcify in a 6 to 8 per cent solution of sulphosalicylic acid.

10. Wash, embed, and so on.

Steps 3 to 7 inclusive are to be performed in the dark.

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2. Antonow, A. Eine einfache Methode für histologischen Kalknachweis. *Ztschr. f. wissenschaft. Mikr.*, 1926-27, 43, 243-245.
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DESCRIPTION OF PLATES

PLATE 36

FIG. 1. Calcifying fibrochondrosarcoma of femoral condyles. $\times 50$.

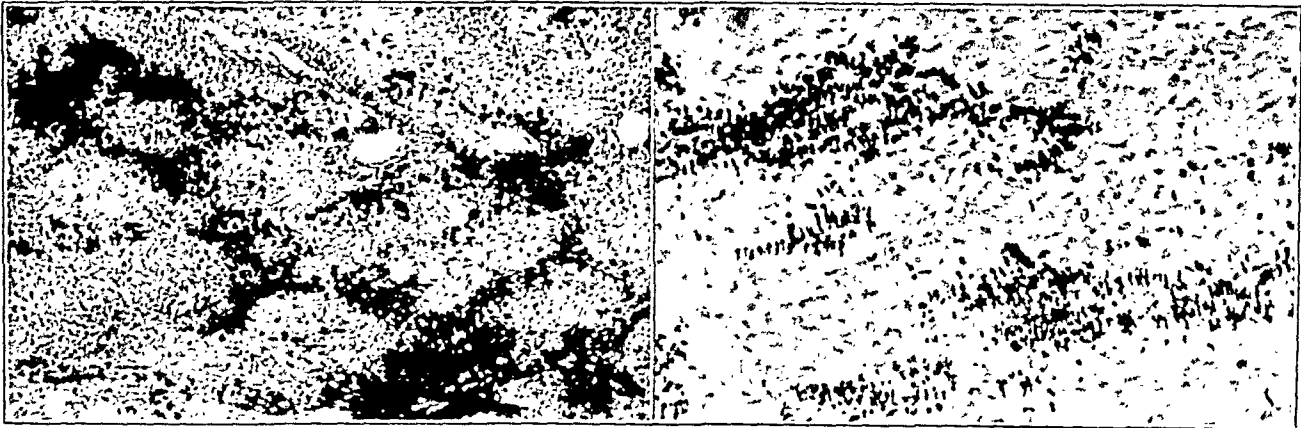
FIG. 2. Another part of the same tumor. $\times 100$.

FIG. 3. Another part of the same tumor. $\times 300$.

FIG. 4. Zone of ossification of lower femoral epiphysis in a newborn. $\times 50$.

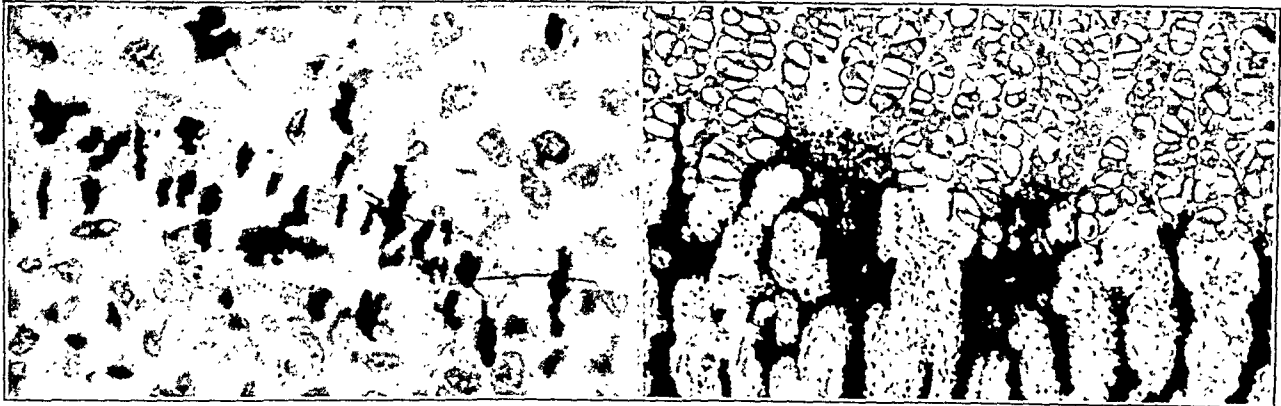
FIG. 5. Sclerotic patch of femoral artery. $\times 120$.

FIG. 6. The part of former figure outlined. $\times 350$.



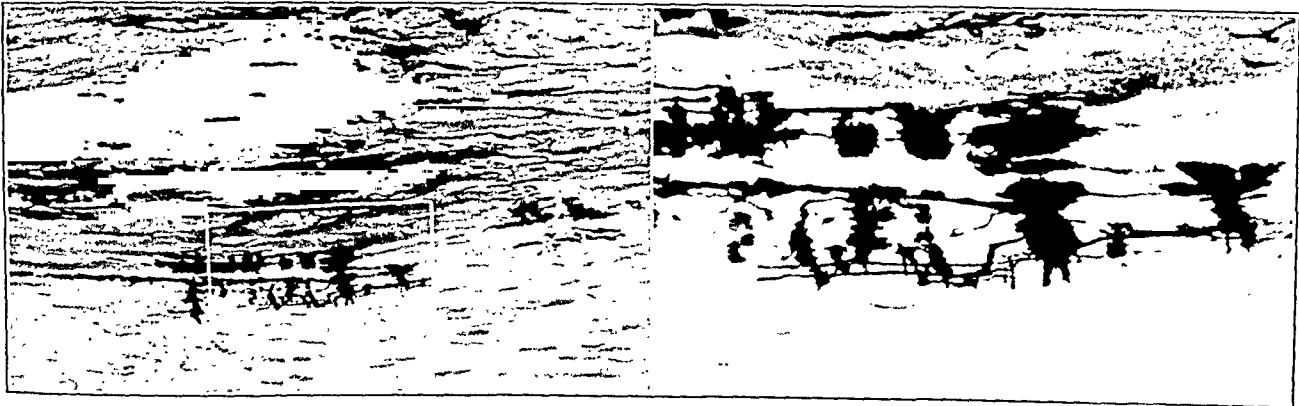
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1000

PIGMENT DEPOSITS IN INTESTINAL MUSCLE COATS AND THEIR RELATION TO DIET FACTORS *

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Some years ago Whipple and Hooper ^{1, 2} observed in a series of bile fistula dogs a peculiar brown or buff coloration of the muscle coats of the intestine. It was described as a "maple sugar color" and was most intense in the jejunum, fading a little in the ileum and still more in the colon. A similar pigmentation involved the stomach wall and pancreas in some cases. No increase in connective tissue was ever observed related to this pigment deposit and no positive stains for iron found. At that time it was suspected that this pigmentation was related to the abnormalities observed by Wisner and Whipple ³ in open bile fistulas. These bile fistula dogs were fed liver in their diet as it was found that this prevented the intoxication so common in the open bile fistulas. No cod liver oil was given to these dogs.

More recently, as Whipple and Robscheit-Robbins ⁴ developed their program to study hemoglobin regeneration in anemic dogs, they observed this brown pigmentation of the muscle coats of the intestine in many dogs that had been fed liver over considerable periods of time. It was suspected therefore that this "maple sugar color" might be due to some dietary factor. The evidence points to *whole liver* in the diet as responsible for the deposit of this pigment in the cytoplasm of smooth muscle cells of the outer and middle muscle coats of the intestine. These observations have a bearing on the origin of pigment deposits of similar nature observed in human disease and old age.

EXPERIMENTAL OBSERVATIONS

Material was available for study coming from a variety of dogs kept anemic by bleeding at a level of 40 to 50 per cent hemoglobin, or one third normal for these dogs. The general care of these ani-

* Received for publication November 29, 1932.

mals, dietary régime and uniformity of living conditions have been described in detail in various publications from this laboratory.⁴ As a rule these dogs are fed during control periods a *salmon bread* which among other things contains cod liver oil. This bread is a complete diet and its preparation has been carefully described elsewhere.⁴ Various food factors may replace or supplement the basal ration of salmon bread and the amount of regenerated hemoglobin and red cells is determined by bleeding the surplus to reduce the dog to the usual anemic level of 40 to 50 per cent hemoglobin. A good many periods of liver feeding occur in the history of these dogs, as careful standardization on liver feeding is done over and over again.

Dog 24-90, with an Eck fistula, had been anemic for approximately 6 years. Age at death 8 years. During the long anemic period in this dog he was given many liver diet periods — a total intake of 26 kilos of various liver material. His liver was injured by chloroform and gradually he lost the capacity to make hemoglobin. He was killed and autopsied at once. The pigmentation of the muscle coats of the small intestine was well established. The stomach and colon showed much less pigmentation in the muscle coats. The pancreas was white. This material was used in the detailed study of this pigment recorded below.

Dog 25-97, a regular member of the anemia colony, was born in our kennels and after weaning was raised on a diet consisting of approximately 50 per cent cooked pig liver and 50 per cent salmon bread plus a milk powder. When full grown the dog was made anemic by bleeding and maintained at a level of 40 to 50 per cent hemoglobin. The usual control periods of salmon bread diet alternated with other diet factors, often liver or liver fractions. This dog in all ingested not less than 36 kilos of pig liver and probably more. The dog lived 5 years and 10 months. The anemia period lasted 5 years. Dog killed and autopsied at once. Pigmentation was a definite brown or buff in the muscle coats of the duodenum. Color faded in the lower small intestine and colon. The muscle coats of the stomach showed definite pigmentation which was much less marked than in the duodenum. This material was used in the tests recorded below.

Dog 30-119 was born in our kennels and after weaning was raised on a diet consisting of approximately 50 per cent cooked pig liver and 50 per cent salmon bread. To the daily diet mixture was added

10 to 15 cc. cod liver oil and 40 gm. whole milk powder. This diet régime lasted 10 months. When full grown the dog was made anemic by bleeding as usual and maintained at a hemoglobin level of 40 to 50 per cent for $7\frac{1}{2}$ months. Age at death 19 months. Total food intake of liver amounted to 21 kilos. Laparotomy was done 3 months before death to fix the spleen to the abdominal wall. At this time it was noted that the small intestine presented a dark brown pigmentation. At autopsy 3 months later the same degree of brown pigmentation was recorded, although no liver had been given in the diet in this interval and the anemia had been continuous. This dog showed no pigmentation of the muscle coats of the stomach or colon. The pancreas was white. This material was used in the tests described below.

Dog 30-118, a litter-mate of Dog 30-119 above, was raised on a diet consisting of approximately 50 per cent raw hamburg steak and 50 per cent salmon bread plus 15 cc. cod liver oil and 40 gm. milk powder. The dog was on this diet 14 months and was anemic at the usual level for 7 months. At autopsy the dog showed no trace of pigment in the intestinal coats, either in gross or in histological preparations.

Dog 30-120, a litter-mate of Dog 30-119 above, was raised on a diet of salmon bread up to 300 gm., milk powder 40 gm. and cod liver oil 15 cc. The dog was on this diet for 14 months and was anemic at the usual level for 5 months. At autopsy the gastrointestinal tract showed no trace of this brown pigmentation, either in gross or in histological preparations.

Dog 27-232 after weaning was raised on a daily diet consisting of salmon bread up to 300 gm., whole milk powder 40 gm., 15 cc. cod liver oil and a salt mixture 2 to 3 gm. The total iron intake was 35 to 40 mg. as Fe daily. The dog was in good health up to a day or so before death. The dog was never made anemic. Found dead. Autopsy showed the cause of death to be infestation with ascaris — the small intestine being full of round worms. The small intestine showed definite pigmentation of the muscle coats. This color was a pale buff or light brown, much less than in the dogs fed whole liver, but the color was definite and not to be mistaken. The salmon bread contained 7 cc. cod liver oil per 300 gm. in addition to the 15 cc. given daily.

GROSS APPEARANCE OF PIGMENT

This pigmentation occurs mainly in the small intestines, shining through the unpigmented serous layer of the intestine, and is apparent immediately upon opening the abdomen. The muscle coats seem to be the only layers involved and the coloration appears to be confined to this particular structure. The mucosa is normal. The pigmentation is usually more concentrated in the upper portion of the small intestine, but occurs also in the large intestine, although here it is of a much lighter tone. The wall of the stomach sometimes shows a faint brown tint. Occasionally the pancreas is pigmented. The pigmentation varies from a light buff to a deep brown, often described as a "maple sugar color." This pigmentation of the intestinal tract is not accompanied by a hyperpigmentation of liver and kidney.

GENERAL MICROSCOPIC DESCRIPTION

Sections of the intestinal tract show that the pigment is found in both the internal and the external muscle layers, but it does not occur in the muscularis mucosae. It appears that occasionally the internal muscle coat contains a greater concentration of this pigment than the external. The color of the pigment is yellowish brown. The pigment is found in small granules in the cytoplasm. In cases of heavy pigmentation the cytoplasm appears densely packed with these granules. If, however, small amounts are found, the granules occur densely packed around the nuclei and rather sparsely in the periphery. These pigment granules generally are of uniform size in the same muscle cell and in unstained frozen sections may appear refractile. Occasionally these granules are found in such a fine form as to be visible only with high power magnification.

REACTIONS TO CHEMICALS AND DYES

It has been observed by Whipple that this pigment is partially soluble in 95 per cent alcohol. Section material placed in alcohol gives up a portion of this pigment to this fluid medium. This solubility may vary, however, in different cases and may be independent of pigment concentration. For example in one animal (Dog 30-119) which showed heavy pigmentation only a small amount was soluble in alcohol, since the fluid was rather light in color even after a period

of 3 months. In some cases showing a less heavy pigmentation the tissues when placed in alcohol gave up a greater portion of the brown color, resulting in a highly colored solution demonstrating varying solubility of this pigment material. This was evident also in paraffin sections, since in some cases of a well marked gross pigmentation sections showed only slight pigmentation. In one case when sections were prepared from tissues kept in 95 per cent alcohol for a period of two months, these sections showed only a slight difference from those prepared from material fixed in formalin. Frozen sections from formalin-fixed material of the same case were made and placed in alcohol in order to determine whether solubility might be increased in a thinner layer of material. These sections were examined at different intervals for a 24 hour period but only a slight fading of the brown color was observed.

Other fat solvents such as ether, chloroform and xylol were tested on sections. Granules were uninfluenced but occasionally a slight amount of color was given off.

Iron reactions were negative in all cases (Berlin blue, Turnbull's blue reactions).

Various fat stains and lipid reactions were tested (Sudan III, scharlach R, Ciaccio, Lorraine-Smith-Dietrich). Results were negative.

Concentrated Sulphuric Acid: The usual yellowish brown color immediately (seconds) becomes much darker and progressively becomes intensified during a 2 hour period. Following this some fading gradually occurs but the original light tones are not attained. Color change is more apparent in frozen sections. (Difference due to previous alcohol treatment of paraffin sections?) No blue coloration on adding acid is noted.

Concentrated Nitric Acid: 20 to 40 minutes following addition of this acid pigment shows slight bleaching. After 2 hours the granules become smaller and partially dissolve. Two to 3 hours later there remain only some undissolved granules in powder form.

Concentrated Hydrochloric Acid: No change following several hours.

Alkali (50 Per Cent Sodium Hydroxide): Granules swell during first 2 hours. Granules in the frozen sections become more refractile. Later the majority of the granules dissolve and there remains only a slight, diffuse brown tint.

Levaditi's Silver Nitrate Method: Granules become dark brown, some black. There are marked differences in different cases. Dog 24-90 showed moderate change, Dog 30-119 numerous black granules. Bielschowsky's method gave the same result.

Equal Parts 1 Per Cent Potassium Ferricyanide and 5 Per Cent Ferric Chloride: Granules become deep blue. The reaction is well marked only in frozen sections.

Hydrogen Peroxide (3 Per Cent and 5 Per Cent Solutions): Two to 3 hours slight bleaching. Fourteen to 16 hours later (observed at intervals) bleaching is more conspicuous — granules are not dissolved but shrunken. After 24 hours bleaching is marked but incomplete and granules are mostly dissolved.

Mounted sections and tissues were exposed daily for several hours to direct *sunlight* during a 5 day period. No effect on pigment.

Basic Fuchsin: In applying the iron reaction to these sections and using Mallory's basic fuchsin solution as counterstain it was noticed that these pigment granules take up the fuchsin. Paraffin sections give satisfactory staining results with basic fuchsin as employed by Mallory to demonstrate *hemofuscin*. For satisfactory staining of the pigment in frozen sections it is necessary to dilute the original basic fuchsin solution approximately 30 times. Staining with dilute solution for 2 to 3 minutes followed by 50 per cent alcohol to differentiate sections mounted in glycerine shows a varying intensity of staining in these pigment granules. Some partially decolorized when in alcohol show the original yellowish brown tint.

Neutral Red (1 Per Cent Aqueous Solution): Staining of frozen sections 2 to 3 minutes (for paraffin sections 8 to 10 minutes) gives an intensive red staining of granules. Sections were rinsed in water after staining.

Brilliant Cresyl Blue (0.5 Per Cent Aqueous Solution): Pigment granules stain intensely deep blue in contradistinction to paler blue with some violet tint of the nuclei. Staining of the granules fades somewhat within 2 to 3 weeks.

Nile Blue Sulphate (Saturated Aqueous Solution): Pigment stains intensely a dark blue, similar in tint but more intense than nuclei. No pinkish violet tint of neutral fat in the granules.

Methylene Blue: Granules show some greenish tint. Probably not a true stain but an admixture of dye and native yellowish brown color of the pigment.

DISCUSSION

Such pigmentation in animals has not been mentioned by other investigators so far as we know. Mallory and coworkers^{5, 6} however observed in experiments with copper administered to rabbits, orally or parenterally, pigment especially in the heart, liver and kidneys which seems not unlike the pigment in these dogs. Mallory compares this pigment to that found in human cases of hemochromatosis. He believes that the pigmentation of his copper-fed animals is identical with the pigment of hemochromatosis. He calls this pigment *hemofuscin* (the name *hemofuscin* was first applied by von Recklinghausen in cases of hemochromatosis) and suggests that it is an intermediate stage between hemoglobin and hemosiderin. Finally it becomes *hemosiderin* in some organs but generally is not found as such in the smooth muscle.

As to origin, classification and nomenclature of this iron-free pigment observed in human hemochromatosis and in old age there exists considerable difference of opinion.

Hueck^{7, 8} names it lipofuscin and believes that the pigment bears a relation to fats and fatty acids and differentiates them sharply from melanins. He based his differentiation upon dissimilarities of staining and microchemical reactions mainly upon the fact that this so-called lipofuscin takes on the fat stains, whereas melanin takes no such stain. Furthermore lipofuscin does not bleach readily with hydrogen peroxide, whereas melanin does.

Lubarsch,⁹ Brahn and Schmidtman^{10, 11} do not differentiate sharply between melanin and lipofuscin, as well as hemofuscin. They believe that all this pigment belongs to the melanin group because they were unable to find sharp differences between chemical reactions as described by Hueck.

Connor¹² investigated the pigmentation of old age, which he calls hemofuscin. His description of the various staining and chemical reactions of this pigment corresponds closely to that found in our dog material. He does not mention the partial solubility of the pigment in alcohol.

Based on the similarity of certain reactions it seems to us that the so-called hemofuscin of Connor, as well as the lipofuscin of Hueck, belong to the same group and may represent a pigment complex.

The dissimilarities of the different chemical and staining reactions may signify a different grouping of radicles within the same complex.

The microchemical and staining reactions of the pigment found in our dogs create an impression that it might be similar to the above mentioned group and represent a pigment complex composed of groups rather than single pigment entities. Our own data and differences observed in different animals as to these various reactions (solubility in alcohol, silver nitrate reaction, fuchsin staining) would strengthen the belief in a pigment complex. The results of microchemical and staining reactions indicate that the pigment found in our dog material may be related to the pigment found in the human material cited above. There is also a similarity in the localization of these pigments in the intestinal tract of our dogs and of human cases which show this pigmentation in the intestine.

Mallory and Connor suggest that this hemofuscin pigment is related to hemoglobin disintegration. In our cases this cannot be admitted and the relation of this pigmentation to diet intake seems established.

We have been unable to convince ourselves that this pigment is related closely to the fats or fatty acids as Hueck claims for lipofuscin.

Our experimental data show that this pigmentation of the muscle coats of the gastro-intestinal tract in dogs is found in anemic and non-anemic dogs, in bile and Eck fistulas, in young and old dogs. One thing alone is constant — that is a large food intake of whole liver or cod liver oil. Whole liver feeding gives a deep pigmentation, while cod liver oil feeding alone may or may not cause a faint pigmentation of the small intestine (Dogs 30-120 and 27-232 above). It will be of interest to observe whether this pigment is found in other animals fed liver and liver fractions. Some pathologist may have opportunity to observe human cases where a prolonged diet intake of liver or liver fractions has preceded death.

SUMMARY

A peculiar brown or buff pigmentation of the muscle coats of the intestinal tract has been observed in dogs. Some of these dogs had bile or Eck fistulae with or without anemia. Other dogs were in the anemia colony of this laboratory and had been continuously anemic

from bleeding for various periods of time. Other dogs were normal. Age was not a factor.

Microchemical reactions and staining properties of this pigment are given. On the basis of these tests one observes some similarity to the pigment observed in human disease (hemofuscin) and in old age.

It is suggested that this pigment is not a definite entity but perhaps a pigment complex. The experiments indicate that this pigment is of dietary origin due to some liver constituent which is absorbed.

Observations on human cases of anemia where large amounts of liver or liver fractions have been administered should prove of interest.

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STRUCTURE SUGGESTING A SPINAL CORD FOUND IN AN OVARIAN DERMOID *

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A year or more ago it was necessary to prepare a class set of microscopic sections, showing the essential structure of the ovarian dermoid, for students to study in the course of instruction in histopathology. Through the kindness of friends and assistants a number of blocks of tissue from various sources were obtained, and after sample sections had been made from them, the one herein to be described was selected. The material is thought to have come from the collection of Dr. Charles C. Norris in the University Hospital, but, as at the time of its collection it was not suspected that reference to the gross material or records would ever be necessary, no identification was kept. Under these circumstances, attempts to trace the block of tissue to its original source have failed, and future attempts seem hopeless.

The material consisted of one block of tissue cut from the "dermoid plug." On each side, and probably on the top, it showed shaggy hairy skin, while the bottom was a smoothly cut surface. Through the center of the block, and passing from end to end, there was a slender cylinder, the length of which cannot now be determined as it had been cut off transversely at each end. It is concerning the nature of this cylinder that we are about to speculate.

When cut sections of the block are examined, the microscopic structure is found to bear out the gross appearance. Skin, with large papillae, hair follicles, sebaceous and sweat glands, is found to make up the greater part, and the whole would have been commonplace had it not been for the presence and peculiar qualities of the cylinder mentioned above, which is composed of nervous tissue.

In describing the "dermoid plug," Frank, in his *Gynecological and Obstetrical Pathology*, 1931, page 401, says: "On inspection of the gross sections, or with a very low magnification, a certain uniformity of arrangement will become apparent. In the most typical

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cases the skin region corresponds to the scalp. Below this, within a fibrous capsule, often reinforced by bone plates, is the brain (pigmented area for eye anlage). In close conjunction are found bone masses with teeth."

The position of the nervous tissue in our section corresponds to that of the "brain" mentioned by Frank, and indeed, it may be a "brain," but its elongated cylindrical form is more suggestive of spinal cord. There are no bony plates homologous with cranial bones, nor is there any area of pigmentation representing the anlage of the eye. The nervous tissue lies in such close juxtaposition to the skin that the hair bulbs appear almost to connect with the membranes by which it is surrounded, as shown in Figure 1.

The configuration of the transverse section of the nervous tissue is strikingly like that of the spinal cord and it is surrounded by a somewhat dense pia arachnoid, although there is no separate dura. There are no definite anterior and posterior commissures, but at several points there is a deep penetration of the membranes, with division of the nervous tissue more suggestive of the commissures of the spinal cord than of the sulci of the brain. There is no central canal, but almost in the center there is a rounded pale mass of neuroglia that may have resulted from abnormal proliferation of ependymal cells.

Under a higher power, the greater part, indeed almost all, of the nervous tissue is found to consist of gray matter, that is, neuroglia containing nerve cells, the only part suggesting white matter being near the center. This arrangement is more like that of the brain with its cortex of gray matter than that of the spinal cord with its H-shaped center of gray matter and peripheral tracts of white matter; but at numerous marginal areas the neuroglia is loose and open, forming a kind of reticulum, as though prepared for penetration by axones, such as would have provided the various tracts of white matter had development progressed along normal lines. Here and there, in the general mass, areas of medullated nerve fibers are observed, but never with such definiteness as to constitute a column or tract. The paucity of white matter might be explained on the assumption that few axones have been formed in the absence of members to be supplied or functions to be performed. Failure of the white matter to develop would also account for the minute size of the cord. The nerve cells are clustered ganglion-wise here and

there, but nothing definite like the columns of Clark appears. Many of the cells give off visible axones.

It is by no means impossible that this nervous tissue is but an unusual form assumed by the "brain" of the "dermoid plug," but its elongated cylindrical form, its appearance in transverse section, its suggestion of commissures and the clusters of ganglion cells, unlike what is seen in the cerebral cortex, suggest that in this case the "brain" has developed in the form of a spinal cord.

In J. Veit's *Handbuch der Gynäkologie*, 1908, 4, Pfannenstiel edits a section, "Die Erkrankungen des Eierstockes und des Nebeneierstockes," of which P. Kroemer, of Berlin, contributes a section entitled "Die Dermoidkystome." On page 230 of this work appears an illustration of a section through a spinal cord that forms part of the structure of an ovarian dermoid. It has surrounding membranes, an elongated, slightly asymmetrical form, a large central canal lined with a single layer of beautiful columnar ependymal cells, and in close relation to it is a large sympathetic nervous ganglion into which a large medullated nerve enters. This is the only occurrence of "spinal cord" in an ovarian dermoid that seems to have been reported.

The probability of the structure observed by Kroemer being a spinal cord is greatly increased by the presence of the central canal, and by the juxtaposition of the nerve ganglion, and that of our case diminished by the absence of both. But a sympathetic ganglion might occur equally well in juxtaposition to brain or spinal cord so that it is not an infallible means of identification, and the large size of the supposed central canal in Kroemer's case might almost as well be interpreted as a ventricular cavity.

It is well known that the various structures in the same dermoid cyst show remarkable inequality of development, some being adult while others are embryonal, and that they are not infrequently overtaken by pathological changes in the course of development. In Kroemer's case the development of the nervous tissue is more advanced, in ours less so; while in ours in the position where the central canal should have appeared, a pathological gliosis seems to have suppressed it.

The difficulty of identification is too great to warrant a definite decision, but the probability that the structure found in our sections is a spinal cord seems sufficient to justify its being recorded as such.

DESCRIPTION OF PLATE

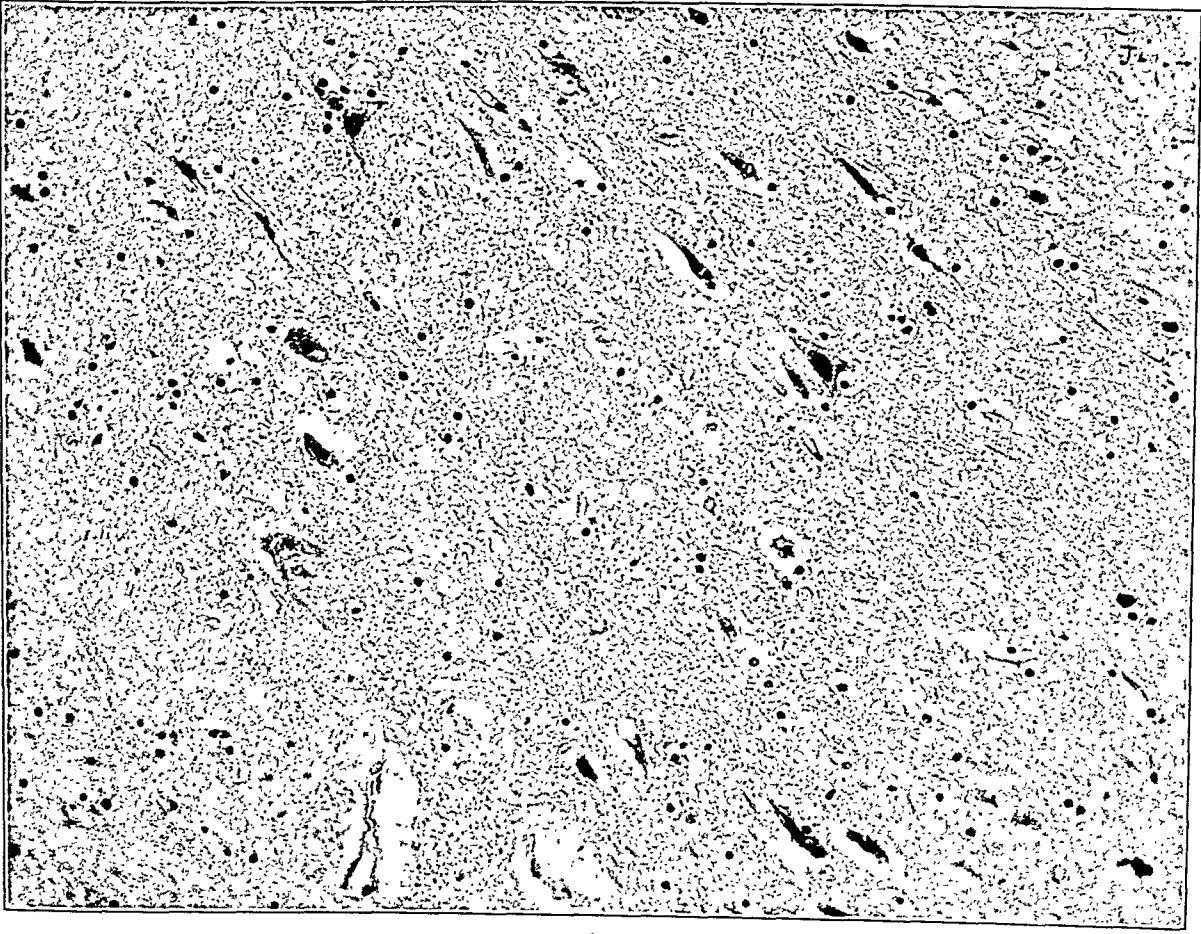
PLATE 37

FIG. 1. General low power view showing the section of the supposed spinal cord surrounded by its membranes.

FIG. 2. Area of a part of the section showing the multipolar ganglion nerve cells, some of which show growing axones.



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THE INCISOR TEETH OF ALBINO RATS AND GUINEA PIGS IN VITAMIN A DEFICIENCY AND REPAIR *

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In 1925¹ we described very briefly two outstanding effects of vitamin A deficiency upon the incisor teeth of white rats — atrophy and metaplasia of the enamel organ and atrophy of odontoblasts accompanied by atypical formations of dentine. Since then we have studied the teeth of vitamin A deficient guinea pigs and added considerably to our understanding of the deficiency changes by the study of the sequences of repair in white rats during recovery induced by the addition of butter fat to the diet. We emphasize the value of following recovery changes in all experimental studies of the consequences of vitamin deficiencies. In vitamin A deficiency, as in vitamin C deficiency (Wolbach and Howe²), cells of mesenchymal origin undergo changes in morphology, making identification inaccurate or impossible. In recovery we have found that such cells, osteoblasts and odontoblasts, resume function before morphological restoration is complete. The presence or absence of a deposit of matrix around cells by way of a recovery response and the character of the matrix if deposited, as well as the restored morphology of the cells, are decisive factors in identification of cells which concern us in studies of teeth in deficiencies.

Few histological studies have been made upon teeth in vitamin A deficiency. May Mellanby has written extensively and wisely upon the importance of vitamin A for the normal development of teeth. These papers fortunately are summarized in an excellent review³

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and present definite proof that defective or absent dentine and enamel formations are consequences of vitamin A deficiency. In her studies of human and experimental animal material, undecalcified ground sections were employed so that cell responses and histological sequences could not be followed. Marshall,⁴ without giving histological details, attributed the formation of "pulp stones" (calcification in the pulp) in white rats to vitamin A deficiency. Coolidge⁵ mentioned and illustrated irregularities of the dentine and the formation of osteoid masses free in the pulp or attached to the dentine in one white rat "of several hundred." The diet of this rat was not given and he gave no histological details, but as the illustrations are those of conditions constant in vitamin A deficiency, we believe that intentionally or inadvertently this rat's diet was deficient in vitamin A. More recently, Shibata⁶ reported as consequences of vitamin A deficiency in white rats "abnormal formation of the enamel, dentin and cementum; degenerative changes such as atrophy and abnormality in enamel organ dental pulp and root membrane tissues; retardation of the eruption of the incisors; and the changes in the chemical compositions such as the decrease of phosphorous and calcium, increase of water magnesium, etc." Shibata refers to earlier papers of his but does not give the references so that we could not consult them for the histological descriptions which the above-mentioned paper lacks. It seems probable that many of the details we shall describe were seen by Shibata.

Our endeavor, as part of a program to achieve specific histopathological characterizations of the avitaminoses, has been to follow the sequences due to A deficiency in the teeth for the purpose of ascertaining the initial or direct (specific) effects, as contrasted with indirect or non-specific effects such as may be the consequences of secondary general disturbances in metabolism. Accordingly sections of decalcified skulls were used. Fixation was either in Zenker's liquid or 10 per cent formalin. Five per cent nitric acid in 70 per cent alcohol was used for decalcifying. Celloidin sections stained with hematoxylin and eosin were routinely used, but various special stains for connective tissue and reticulin were employed when indicated.

The diet used for the rats was that described by us in 1925. Whether or not the absence of vitamins C, D, and E was of importance we cannot say. We adhered to it because (1) scorbutic effects cannot be produced in rats; (2) vitamin D is not necessary to prevent

rickets in rats with properly balanced inorganic salts in their diets when kept under proper hygienic conditions; (3) we know of no effects of vitamin E deficiency outside of the testes in the male and the products of conception in the female; and (4) of greatest importance, the addition of vitamin A alone sufficed to bring about histological recovery.

The diet used for the guinea pigs was that described by us in 1928.⁷ We varied the amount of orange juice in an attempt to find an amount which would prevent scorbutus and yet allow an A deficiency. Twenty cc. of orange juice daily is sufficient to prevent histological effects of vitamin A deficiency in any organ in guinea pigs of 300 to 500 gm. weight. Eight cc. daily does not prevent the epithelial metaplasia of vitamin A deficiency. Two cc. daily is sufficient to prevent scorbutic lesions in bones. The majority of our guinea pigs received 4 to 8 cc. of orange juice daily. This we emphasize because certain findings in the teeth of guinea pigs which were reported by Höjer⁸ are apparently duplicated in guinea pigs on vitamin A deficient diet plus 4 to 8 cc. of orange juice daily.

Our studies were made from frontal plane sections through the skull at three or more levels each, including the upper incisor teeth. Owing to the curvature of the incisor tooth the frontal plane sections do not give true cross-sections, except in the midportion of the tooth. Allowance must be made for this in viewing the illustrations because the sections through anterior and posterior portions of the tooth cut the labial and lingual walls obliquely. The lower incisors were studied in sections at the level of the first molars in rats with different degrees of the deficiency. In guinea pigs and in rats during recovery the lower jaws were sectioned at three levels, one through the first molars, and two posterior to that.

The following tables serve to indicate the scope of our material.

THE INCISOR TEETH OF RATS

The gross changes have not been accurately followed by us. Growth of the teeth seems to be commensurate with the growth of the skull, which shares in the marked retardation of the skeleton as a whole. The teeth lose the normal orange pigmentation and acquire a chalky-white appearance, due we believe to loss of the enamel which is the pigmented part, and to change in composition of the

TABLE I

Vitamin A Deficient Rats Used for Tooth Studies

No. of rat	Duration of deficient diet		Metaplasia all organs
	<i>days</i>		
227.....	61	Died	+
224.....	63	"	+++
207.....	77	"	++
209.....	82	"	+
205.....	89	"	++
206.....	89	"	++
220.....	91	"	+++
222.....	97	"	+++
203.....	98	"	++
201.....	100	Killed	++
202.....	101	"	+++
238.....	114	"	++
101.....	115	"	+
31.....	149	Died	++++
35.....	149	Killed	++
105.....	160	Died	+
107.....	167	"	+
106.....	171	"	+
103.....	180	"	++++
100.....	181	Killed	+

TABLE II

Vitamin A Deficient Rats in Recovery Used for Tooth Studies

No. of rat	Deficiency period	Repair period	Meta-plasia of epitheliums	Weights in grams			
				Initial	Maximum	End of deficiency	Recovery
	<i>days</i>	<i>days</i>					
212.....	70	9	++	95	128	105	?
239.....	103	5	++	63	123	99	?
425.....	118	10	+	141	162	147	144
232.....	137	6	+	114	152	120	?
235.....	138	5	+	104	140	126	85
397.....	138	12	+	84	136	119	135
393.....	143	18	+	85	128	109	125
394.....	146	22	+	81	119	80	105
413.....	150	13	++	135	136	104	107+
399.....	151	15	+	86	140	123	142
400.....	151	20	++	81	129	107	129+
401.....	161	22	++	81	114	87	117
412.....	170	14	++	128	137	126	169
414.....	170	19	+++	143	146	112	160
50.....	185	43	++	?	?	?	?
417.....	187	7	+++	133	133	102	120

In the last column of weights + means that the recorded weight was that of several days previous to killing.

TABLE III

Vitamin A Deficient Guinea Pigs Used for Tooth Studies

No. of guinea pig	Duration of deficiency	Metaplasia all organs	Orange juice daily
	<i>days</i>		<i>cc.</i>
2.....	66	+	2
28.....	67	++	8-2*
29.....	67	++	8-2*
24.....	71	++	20-2*
38.....	101	?	16-4
23.....	105	+++	20-4-0**
42.....	105	+	16-8
39.....	110	+++	16-2
6.....	112	?	20-8
142.....	128	+++	4
45.....	144	?	20-8
15.....	153	++	20-4
46.....	158	?	20-8
143.....	160	++	4
72.....	161	++	16-2
8.....	163	++	20-8
30.....	287	0	20
41.....	299	++	20-5

* These three guinea pigs, after the orange juice was reduced to 4 cc. daily, received none for 2 weeks and then received 2 cc. daily for the final 2 weeks of life.

** This guinea pig received no orange juice for the final 2 weeks of life.

dentine. Also, the teeth are more brittle than normal. Striking histological changes were present in all the teeth of the rats listed in Table I so that a pronounced effect resulting in change of cross-sectional shape is apparent in from 60 to 70 days, possibly earlier. At all levels we find the dentine on the labial side of the tooth disproportionately thick, compared to the lingual, lateral and mesial sides. Figures 1 and 3 are typical of A deficiency. In advanced deficiency (Figs. 3 and 4) there may be gaps in the wall of dentine on either side, but always near the basal, open end of the tooth. An early effect of the deficiency is atrophy and cessation of function of the odontoblasts. The odontoblasts on the labial side (adjacent to the enamel organ) are affected last and this accounts for the final cross-sectional shape of the tooth. We have not determined the rate of growth of the incisors in vitamin A deficiency. In the normal albino rat (Addison and Appleton ⁹) the rate of growth of the upper incisors is given as 2.2 mm. per week, of the lowers, 2.8 mm. per week.

Addison and Appleton also give the following lengths in millimeters of the incisors at different ages.

Age	Upper incisors	Lower incisors
23 days	12.8	18.1
41 "	15.0	21.7
10 weeks	18.3	25.5
15 "	20.3	26.4
5 months	23.3	29.4
8 "	23.7	29.9
10 "	26.2	31.3

If in vitamin A deficiency the *rate* of growth of the incisor teeth in *relation* to the growth of the skeleton as a whole is approximately that of the normal animal, it is probable, and we believe certain, that the entire tooth is replaced through growth during dietary periods covered by our observation. This must be kept in mind in studying sections taken at different lengths from the formative end of the tooth.

The enamel organ which extends for the entire length of the incisor tooth on its labial surface (elsewhere only about 1 mm. from the basal formative end) early undergoes atrophy and metaplasia (Fig. 2) and consists finally of atrophic remnants of the epithelial papillae and squamous cells replacing the ameloblasts and stratum intermedium (see Fig. 5 for contrast). The anterior part of the enamel organ is first affected but finally the whole length is involved, including the basal formative end (Rat 31, Table I). At the basal end there is continuous renewal of the enamel organ and in many rats with marked metaplasia in many organs practically normal structures were found here, though with distorted relationships, owing to pressure deformity of the tooth because of defective or absent dentine formation. According to Addison and Appleton the ameloblasts travel forward as the incisor grows and it is a fact of interest that for a brief period these cells, as well as the rest of the enamel organ, may retain normal structural appearances. The effect of the deficiency is most apparent after the ameloblasts have reached the region where they are normally most active in enamel formation. Toward the occlusal end, though still within the area of functional enamel organ, the metaplasia may become complete and as a result the enamel organ may be replaced by an epidermis-like structure with superficial keratinized cells. In middle and posterior parts we have not seen this degree of metaplasia. In the atrophy of the enamel organ (Fig. 5) the epithelial papillae are affected first. These structures shrink in size because of atrophy of cells and the surrounding connective tissues become less vascular. The ameloblasts diminish in size, become

coarsely granular and finally disappear. Cell outlines are lost and the ameloblast layer becomes represented by a layer of granular material and pyknotic nuclei, and then disappears. Cells presumably of the stratum intermedium persist as flat cells, usually two rows deep, until in very late stages in the anterior half of the tooth these develop numerous layers of flat cells, the most superficial of which occasionally become keratinized. Very rarely, in contrast to its occurrence in guinea pigs, after complete loss of ameloblasts globular formations of calcified material develop in the connective tissue adjacent to atrophied papillae (Fig. 6). This calcification following atrophy of the structure whose function it is to receive and segregate calcium salts from the blood stream, invites speculations regarding the possibilities of selective permeabilities of the capillaries supplying the epithelial papillae.

The sequences determined by study of rats in varying degrees of the deficiency are supported by the sequences of repair during recovery induced by feeding butter fat. The first evidence of recovery in the enamel organ is the plumping of cells of the epithelial papillae. This is accompanied by engorgement of capillaries and a less dense appearance of the connective tissue immediately adjacent to the papillae. The earliest response was in Rat 417 (Table II) after 7 days of treatment. Subsequently, and in a very few days, the surface cells, presumably of stratum intermedium origin, become cuboidal in shape and continue to assume more and more the appearance of ameloblasts (Rats 397 and 399, Table II, for example). In rats responding favorably to treatment, as shown by rapid gain of weight, the enamel organ is practically normal in appearance on the 19th day (Rat 414, Table II).

The details of changes in the pulp and odontoblasts are more difficult to follow than those in the enamel organ. Simultaneously with changes in the enamel organ occurs atrophy of the odontoblasts but, as we have said above, the odontoblasts on the labial side (adjacent to the enamel organ) retain their morphology and function for long periods. This accounts for the extraordinary thickness of the dentine on the labial side, as contrasted with the remainder of the tooth. It is naturally a pertinent suggestion that the enamel organ, even though undergoing atrophy and separated by a wall of dentine and enamel, still exerts an influence upon the maintenance of the odontoblasts comparable to that in the fetus and at the basal formative end.

Conversely, one may argue that all of the changes in the odontoblasts are secondary to atrophy of the enamel organ and withdrawal of its influence. At the basal formative end the odontoblasts undergo early atrophy, except on the convex or labial surface.

The atrophy of odontoblasts, with the exception of those opposite the enamel organ, proceeds rapidly and is complete usually when general histological changes in many organs are well established, and therefore in as short a period as 60 to 70 days. All cells resembling odontoblasts disappear and the internal surface of the dentine becomes bounded by cells that are indistinguishable from other connective tissue cells of the pulp until extreme thinning of the dentine has resulted (Fig. 7).

During the process of atrophy, odontoblasts, and rarely capillaries that supply them, become incorporated into the dentine because some cells remain functional for a longer time than others and by continuing to form it surround non-functioning cells with dentine. This detail is most strikingly seen when the odontoblasts on the labial side finally begin to atrophy (Fig. 8).

In rare instances, not accounted for by us, cells resembling osteoblasts in size and shape were present on the inner surface of the dentine and had deposited a thin interrupted layer of bone (secondary dentine?).

In the depths of the pulp small areas of osteoid tissue were frequently found in rats with fully developed deficiency. Going over the whole series of rats we worked out the sequences of pulp ossification as follows: first, minute spherical globules of hyaline matrix appear between the pulp cells and simultaneously narrow zones of similar material form about occasional capillaries; these deposits increase and incorporate pulp cells which assume the morphology of osteoblasts, and finally cells in contact with the periphery acquire the morphology of osteoblasts and presumably function as such.

In consequence of the atrophy of odontoblasts, as the tooth continues to grow, the dentine near the formative end becomes very thin, often sharply folded or pleated, or entirely absent (Figs. 3, 9, 10 and 13). Associated with these late consequences of the deficiency are aggregations of cells of two types, each responsible for the formation of masses of dentine (denticles) continuous with or separated from the tooth wall. In every instance where our sections passed through or near to the basal formative end, when these cell aggrega-

tions were present we found strikingly defective dentine formation associated with atrophic odontoblasts and much atrophied ameloblasts in Hertwig's sheath (the enamel organ at the basal formative end).

In some instances columnar cells were enclosed within folds of dentine and the relationships were such as to indicate positively that these cells were incorporated ameloblasts. The morphology indicated also that these cells were ameloblasts because they were cylindrical and radially arranged in contrast to the shrunken odontoblasts on the opposite side of the dentine. In regions of complete absence of the dentine at or close to the formative end there were frequently, in the gaps, gland-like clusters of cells that we are also forced to interpret as of enamel organ origin. Such gland-like formations also lie under the thin abnormal dentine (without canaliculi) at considerable distances from the basal end (Figs. 11, 12 and 15). One type of cell aggregate then we regard as of enamel organ origin and due to the fact that cells (presumably ameloblasts because, as we shall show below, they do not survive in repair) become incorporated by the pleating of a very thin and atypical dentine at the formative end.

The other type of cell aggregate is proliferative in type and consists of cells derived from the pulp that forms in regions of extremely thin or totally absent dentine. These cells are short, plump, basic staining, and resemble transitional forms between mesenchymal cells and osteoblasts. Their presence is accompanied by deposition of atypical dentine (osteodentine) upon the tooth wall (Figs. 3, 9, 10 and 11), although in very severe deficiency they form no matrix at all. These cell aggregates we regard as reparative in origin, comparable to callus formation in bone, and indicative that even in extreme vitamin A deficiency proliferative responses on the part of connective tissues may be energetic. We have further proof of this in the repair of wounds made for experimental purposes. All of our decisions regarding origins of cells were made after careful study of the histology of repair during recovery induced by substituting butter fat for lard in the diet.

An outline of the sequences observed in recovery is therefore indicated. As we have described above, the restoration of the enamel organ takes place first at the basal formative end. Very promptly the odontoblasts in juxtaposition to the enamel organ at this end recover and by the 7th day (Rat 417, Table II) in the region of Hert-

wig's sheath they have assumed normal morphology and function, as shown by the deposition of predentine with canaliculi. The predentine calcifies rapidly. Folds in pleated dentine fill in rapidly. Excess atypical dentine, so-called secondary dentine or osteodentine, the product of the proliferative pulp cell aggregates and denticles of ameloblast inclusion origin grow in size by the deposition of dentine upon them (Figs. 16, 17 and 18). The ultimate fate of these "denticles" we have not followed, but it seems reasonably certain that they continue to increase in size while advancing forward as the tooth grows.

The reappearance of columnar odontoblasts is represented in all stages by our series of repair experiment rats. It takes place first at the basal formative end and works forward, first upon the labial side of the tooth, presumably in response to the recovery of the enamel organ. Later it extends throughout the tooth. Predentine formation is active before the odontoblasts have acquired columnar shapes. Complete morphological recovery was almost achieved in 19 days in Rat 414, which showed a very satisfactory increase of weight in response to butter fat in the diet. There is little to record about the sequences of odontoblast recovery. The changes observed at different periods of recovery were quite like those to be seen in the formation of osteoblasts in granulation tissue in the repair of bone. Connective tissue cells in contact with the dentine become plumper and more basic in staining reaction. For a brief period they are polyhedral in shape and processed like young osteoblasts. Soon they become aligned more or less parallelly with their long diameters oblique or perpendicular to the dentine, but before there is any regularity in arrangement or uniformity in shape, predentine is deposited upon the old dentine. The sequences may be arranged in four stages: (1) increase of size, or at least of cytoplasm concentrated about the nucleus; (2) assumption of osteoblast-like morphology; (3) evidences of function and polarity, as evidenced by directional deposit of predentine; and finally, (4) further growth in size and acquisition of the normal shape.

Various stages of odontoblast recovery may be seen in one tooth because, as stated above, the recovery begins first opposite the enamel organ, *i. e.* at the basal end and labial surface. We have thus had ample opportunity in our recovery series to feel secure in com-

paring the recovery or reappearance of odontoblasts to the sequences seen in bone repair from fibroblast to osteoblast. Our observations lead us to regard, from the morphological viewpoint, the odontoblast as a polarized osteoblast.

The recovery sequences permit no doubt regarding the nature of the proliferative cell aggregates. They are of pulp connective tissue origin. The other types of cell aggregate in gland-like formation we have already concluded were of enamel organ origin. Again the morphological evidence is supplied by the sequences seen in recovery. The first change in recovery is an increase in size of the cells, some of which undergo mitosis (Fig. 12). The pulp cells in contact with these gland-like formations *respond, as do those in contact with the dentine*, and proceed to deposit predentine. We thus get the picture of epithelial cells in the pulp surrounded by dentine, in turn surrounded by odontoblasts. This is shown in low power at A and B in Figure 13 and at higher power in Figure 14. The enclosed epithelial cells undergo atrophy and disappear (Fig. 19). The last stage of degeneration is like that of the ameloblasts in the deficiency. The cells break up into coarse granules and the residue becomes calcified; pyknotic nuclei can be seen in denticles of this origin for some time after calcification of the cell débris (Fig. 18).

Excessive local formation of atypical dentine (denticles) is produced in vitamin A deficiency by focal proliferative responses of pulp cells. Such deposits are insignificant, but increase in the recovery period commensurate with rate and extent of recovery. Another type of excess dentine formation or denticle is initiated by the presence of cells of the enamel organ that become incorporated by folds of dentine or through total gaps in the dentine. In repair these cells stimulate the formation of odontoblasts from the adjacent pulp and hence denticle formation. We thus have brought new experimental evidence to support the prevailing opinion (Orban¹⁰) that cells of Hertwig's sheath may be carried forward into the pulp and stimulate the formation of odontoblasts from pulp cells, and consequently the formation of denticles. Fridrichovsky¹¹ described in human teeth and in rats' teeth (diet not stated) folds of dentine such as were of common occurrence in our series. These he regarded as due to developmental irregularities of Hertwig's sheath. He expressed the opinion that such folds could carry enamel organ cells

forward, and thus accounted for one source of denticle formation. Naturally he did not consider deficiency disease as a possible cause of the mechanism.

THE INCISOR TEETH OF GUINEA PIGS

In general the effect of vitamin A deficiency upon the incisor teeth of guinea pigs is similar to that in rats. There are some quantitative differences and some apparent contradictions appear to our interpretation of events from the teeth of rats. The outstanding difference is the rapidity and severity of the enamel organ changes. The apparent contradiction is exhibited in the prolonged persistence of the morphology and function of odontoblasts after marked degrees of enamel organ atrophy.

The durations of the deficient diet in the experiments with guinea pigs were on the whole shorter than those with rats, inasmuch as at the beginning the majority of the guinea pigs received sufficient orange juice to afford very considerable amounts of vitamin A. Twenty cc. of orange juice daily is completely protective against histological vitamin A effects in all tissues, except occasionally in the respiratory mucosa in the nares. Sixteen cc. daily is nearly protective. Therefore, the durations given in Table III are not those of completely deficient diets. For example, Guinea pig 38 received 16 cc. of orange juice daily for 56 days, 8 cc. daily for 21 days and 4 cc. daily for 24 days. Guinea pig 15 received 20 cc. for 6 days, 8 cc. for 90 days and 4 cc. for 57 days. Guinea pig 42 received 16 cc. for 55 days, 8 cc. for 50 days; Guinea pig 39 received 16 cc. for 63 days, 4 cc. for 7 days and 2 cc. for 40 days. Guinea pig 6 received 20 cc. for 70 days and 8 cc. for 39 days. Guinea pig 45 received 20 cc. for 69 days and 8 cc. for 75 days. Guinea pig 46 received 20 cc. for 28 days and 8 cc. for 130 days. Guinea pig 8 received 20 cc. for 52 days and 8 cc. for 11 days. Guinea pig 41 received 20 cc. for 209 days, 10 cc. for 15 days and 5 cc. for 75 days.

Gross evidence of scorbutus was absent in each of the guinea pigs in Table III. Histological evidence was found in the costochondral junctions of Guinea pig 23 which received no orange juice during the last two weeks of the experiment. These changes were so slight as to suggest that the retardation of growth secondary to the A deficiency was a factor, inasmuch as there was the typical athreptic type of

costochondral junction. In Guinea pigs 24, 28 and 29 there were microscopic evidences of repaired scorbutus in the teeth, such as we described in 1926.² The scorbutic factor, as possibly responsible for the changes we found in our vitamin A deficient guinea pigs, may be dismissed because of the fact that all of the changes we are describing were found in guinea pigs that had never received less than 8 cc. of orange juice daily and that exhibited no gross or microscopic evidences of scorbutus. This we emphasize because Höjer⁸ has described and illustrated changes involving odontoblasts and dentine, with the production of osteoid or bony formations in the pulp and upon the dentine in latent or partial scorbutus. As our own work with scorbutus has been upon animals completely deprived of vitamin C we have as yet no basis for criticizing Höjer's conclusions. Such premises as we now have incline us to believe that in partial vitamin C deficiency Höjer's results would be duplicated. Both vitamin A and vitamin C deficiencies affect odontoblasts, though by different routes, but the effect upon dentine formation may be the same.

THE ENAMEL ORGAN

The first demonstrable effect of the deficiency upon the incisor teeth is in the enamel organ. As in the case of the rat the external layer atrophies first. The ameloblasts become smaller and shorter. In rapidly progressing deficiency they finally undergo granular degeneration and disintegrate. Usually low cuboidal cells persist for long periods and we are not able to say positively whether they are atrophied ameloblasts or cells of the stratum intermedium, though we believe they are derived from the latter, inasmuch as often these cells have horizontal diameters nearly double the vertical, and it is improbable that atrophied cells should undergo such a change. Eventually the enamel organ completely disappears, unlike what occurs in rats. Before the atrophy is complete globules of calcified matrix appear in the outer layer of the enamel organ between the inconspicuous epithelial papillae and within the epithelium itself. These globules increase in size and number, extending into the adjacent connective periodontal membrane. Similar calcified deposits form above the atrophic remains of the enamel epithelium and enclose epithelial cells (Figs. 20 and 21). When large, these calcified deposits take on the appearance of bone (Figs. 22 and 23), because

of the incorporated cells. However, the cells do not have the appearance of osteoblasts. They are without processes and in the largest plaques are surrounded by clear spaces reminiscent of cartilage rather than bone. Neither do these plaques become surrounded by osteoblasts. They resemble cementum rather than other examples of pathological bone formations, such as we are familiar with. They are often bounded on the deep side by cuboidal epithelial cells that laterally are continuous with atrophic cells of the enamel organ. The plaques increase by accretion. Globules of matrix continue to be deposited from the adjacent connective tissue.

THE ODONTOBLASTS

Atrophy of the odontoblasts occurs late. In no guinea pigs of our series was there complete cessation of dentine formation; always there were on the labial side a normal-appearing layer of odontoblasts and evidences of functional activity; on mesial and lateral sides only partial atrophy was found and hence the cross-sectional shape peculiar to the rat's incisors was not seen in the guinea pig. As in the case with rats the atrophy of odontoblasts appeared first on the lingual side of the tooth and never before advanced atrophy of the enamel organ had occurred. A very striking difference from occurrences in rat teeth is that coincident with the atrophy of odontoblasts, spicules of a modified dentine form (Fig. 24). This material, at first uncalcified and similar to predentine in all respects except that it contains no canaliculi, becomes finally calcified and continues to grow by peripheral deposit. In long-continued deficiencies, when these dentine-like deposits extend deeply into the pulp, they become surrounded by osteoblasts which they incorporate as they grow; further increase in size is like that of growth of osteoid tissue (Fig. 25).

The earliest formation of these amorphous dentine processes we found in regions of most marked atrophy of the odontoblasts and always upon the lingual side of the tooth. The initial stage we found to be a deposit of predentine-like matrix between atrophied odontoblasts that had lost their columnar form. It was evident that functional activity continued, but polarity of the odontoblasts was lost. In early formations but few cells are found enclosed in matrix. The outgrowths for a period are surrounded by other atrophic cells of the

odontoblast layer which retain their polarity. Finally, polarity is lost and the cells concerned in further growth are indistinguishable from osteoblasts.

The Pulp: The pulp remains practically unchanged. The only ossification we found in our series took origin in the manner described above as a continuation of growth of dentinoid processes.

The Cementum: As a rule in well established A deficiency there are local increases in thickness of the cementum, for the narrow, deeply calcified border surrounding the dentine is to be regarded as cementum. In fact the only cementum resembling bone we have seen in connection with the incisors of guinea pigs has been in A deficiency animals. The formation of cementum of appreciable thickness and enclosing cells is always found first upon the lingual side, but may extend on lateral and mesial sides forward to the enamel organ region as an irregular layer (Fig. 24). Frequently sharply circumscribed cementum formations were found, perhaps warranting application of the term cementicle (Fig. 26).

DISCUSSION

Three facts are of assistance in interpreting vitamin A deficiency effects upon incisor teeth of rodents: (1) the enamel organ moves forward with the growth of the tooth so that the ameloblasts maintain throughout the same relative position to calcified structures; (2) the dentine increases in thickness progressively toward the anterior (occlusal) end, indicating activity of the odontoblasts extending nearly to the end of the tooth; and (3) the occlusal end of the pulp chamber is being constantly filled in by bony material (osteodentine), indicating a normal tendency on the part of pulp cells to produce osteoid matrix and cessation of a polar deposition of matrix on the part of the odontoblasts.

Fact 1 is of assistance in explaining why the earliest and maximum atrophy of the enamel organ is found at some distance from the formative end as a requirement of time for the deficiency to take effect. Fact 2, as dentine is not resorbed in consequence of the deficiency, explains the greater thickness of dentine for long periods in the deficiency toward the occlusal end. Fact 3 warrants the assumption that the deposit of osteodentine following atrophy of odontoblasts is an adaptation or premature stimulation of a normal sequence.

The enamel organ changes we have described in rats and guinea pigs are primary consequences of vitamin A deficiency common to many epithelial organs. The more rapidly produced and pronounced changes in guinea pigs, as compared with rats, are in conformity with the changes in other organs in these two animals (Wolbach and Howe¹).

We prefer to interpret some effects upon odontoblasts as secondary to enamel organ atrophy, assuming that the latter, in teeth that are continuously growing, exerts an influence upon the former, as suggested by the early sequences in tooth formation and throughout life at the basal formative end of rodents' incisors. This interpretation is not supported by the facts that odontoblasts persist after disappearance of the enamel-forming organ in all stationary permanent teeth of animals, and that in incisor teeth of rodents the enamel-forming organ is continued forward only upon the labial side. Nevertheless, our observations show that enamel organ atrophy precedes atrophy and depolarization of odontoblasts. The fact that in rats odontoblasts remain morphologically and functionally active on the labial side, in apposition to the enamel organ, long after complete disappearance upon other surfaces, and that in this situation their eventual atrophy only follows enamel organ atrophy, demands the above interpretation. The finer sequences attending atrophy of the odontoblasts on the labial side, which we have carefully followed, here and there an odontoblast ceasing to deposit matrix upon its external pole, with continued deposition centrifugally, support strongly the argument that even though the odontoblast may be individually physiologically independent of the enamel organ, architecturally it is dependent. We have come to regard the odontoblast as a polarized osteoblast and the enamel organ as the polarizing agent. In guinea pigs the same changes occur, though in less evident degree. The persistence of normal odontoblasts upon the labial side is more pronounced than in rats. Odontoblast atrophy is less striking, yet illustrates more conspicuously in the sequences we have described the effects of the depolarization of odontoblasts, as shown by the extensions into the pulp of the osteodental processes (denticles) built up by these depolarized cells.

Late vitamin A deficiency effects in bones, as described by Pappenheimer,¹² and completely confirmed by us in unpublished studies, include complete cessation of growth. It may, therefore, be argued

that effects similar to those in bone should be expected in odontoblastic activities and that both are of the same nature. This we may admit without affecting the rôle of the enamel epithelium in the determination of the order of events. More direct attack based upon experimentally produced lesions of the enamel organ is needed to settle these problems.

The formation of bone in rats in the pulp proceeds, we have learned, as does the ossification in membranous bone formation, through direct change in character of the intercellular matrix, preceded by finely granular calcium deposition. Calcification in and adjacent to the atrophied enamel organ, followed by ossification, in guinea pigs may be regarded as a phenomenon having similar pathogenesis. In both instances the cells directly responsible for the elaboration of specific calcified structures, dentine and enamel, are partly or completely inactive, a fact that invites speculations along the lines of a continued extracapillary delivery of calcium and phosphorous compounds to the tissues, and therefore to questions of differentiated permeability of capillary endothelium. The high mineral content of enamel and its rapid growth rate present problems concerning concentration that make such speculations attractive.

We have presented two origins of osteodentine excrescences, or denticles, one occurring in both rats and guinea pigs by the depolarization of odontoblasts, and in the rat accompanied by a proliferative reparative response of pulp cells; the other, only in rats, induced by inclusions of ameloblasts in folds of recently formed dentine, late in the deficiency, close to the formative end. These solutions as to origin could not have been achieved without having for study many stages of repair.

We have observed no changes in the exceedingly thin calcified zone surrounding the dentine in rats which by some morphologists is regarded as cementum. In guinea pigs definite cementum overgrowths (cementicles) were frequently seen on the lingual side of the teeth in consequence of the deficiency, but we can offer no explanation except the suggestion that they formed in response to structural weakness of the bony socket as a part of the general athreptic skeletal effect of late vitamin A deficiency.

The application of our observations to human teeth obviously must be restricted to the formative period and particularly to teeth of the second dentition. Our inference, based upon studies of experi-

mental scorbutus,² and rickets (unpublished), is that vitamin A deficiency is the most important because of its effect upon the enamel organ and because recognition of this deficiency is always late and will continue to be so, unless other criteria than ocular effects are looked for by pediatricians. Defective enamel formation, ossification of the pulp, denticle and cementicle formation are all possible consequences of vitamin A deficiency in humans. Studies of unerupted teeth from infants in vitamin A deficiency, obtained post-mortem, are being made by Boyle,¹³ and he has recorded observations that indicate we may safely apply some of our observations to the human.

SUMMARY AND CONCLUSIONS

1. The initial effect of vitamin A deficiency upon incisor teeth of rats and guinea pigs is upon the enamel organ. The ameloblasts respond earliest by atrophy, then the remainder of the organ atrophies; finally metaplasia and calcification, and, in the guinea pig, ossification occur.

2. Atrophy and depolarization of odontoblasts follow enamel organ changes. The odontoblasts survive longest on the side (labial) where in apposition to the enamel organ and in long-continued experiments gross deformities in the incisors of rats resulted from absent or deficient dentine formation.

3. Two types of denticle formation are described, one built up by depolarized odontoblasts, the other by inclusions of ameloblasts by the folding of imperfectly formed dentine at the formative end of the tooth.

4. Defective enamel formation and other poorly understood conditions in teeth, such as denticles, pulp bone and cementicles, may reasonably be regarded in the human as vitamin A deficiency possibilities.

5. Our observations indicate that in the incisor teeth of rodents the odontoblasts throughout life are influenced by the enamel organ.

6. As in other morphological problems concerning vitamin deficiencies, study of the sequences of repair was essential. We emphasize the importance of two types of control material, the normal and progressive stages in repair.

7. Our observations indicate that vitamin A deficiency is the most important of the known vitamin deficiencies in its effect upon tooth formation.

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DESCRIPTION OF PLATES

PLATE 38

Fig. 1. Rat 201. Upper incisor, typical of A deficiency in the rat. 101 days on vitamin A deficient diet. Atrophy and metaplasia of the enamel organ. Odontoblasts still present on labial side where the dentine is abnormally thick. Note thinness and folding of dentine on both sides of tooth where there are also many large areas of cellular proliferation derived from pulp cells and possibly from odontoblasts. Osteoid formation present in pulp. $\times 46.2$.

FIG. 2. Rat 201. High power of enamel organ. Same preparation as Fig. 1. 101 days on vitamin A deficient diet. Note great atrophy of the epithelial papillae, absence of ameloblasts and the several layers of flattened cells upon the surface. $\times 588$.

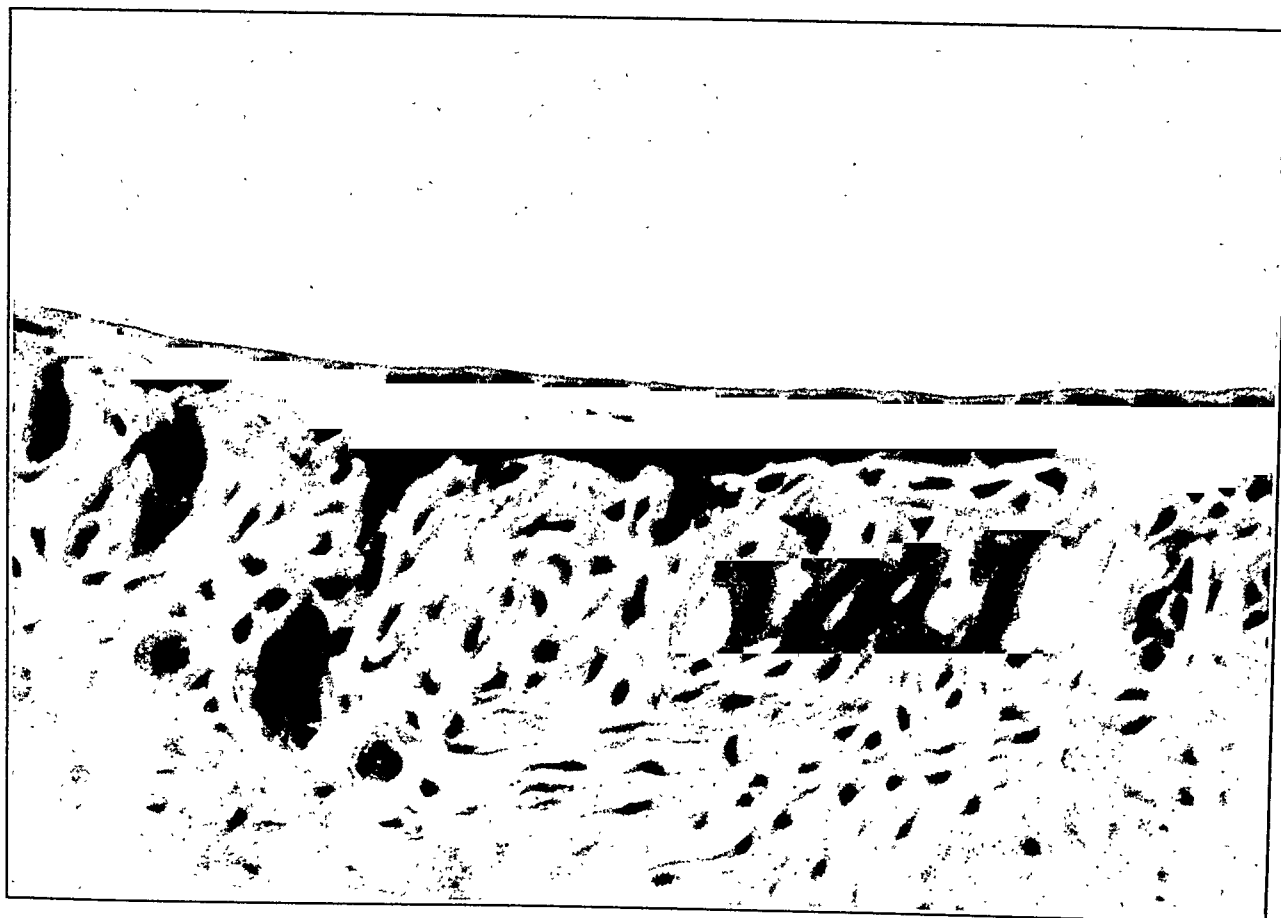


PLATE 39

FIG. 3. Rat 205. Upper incisor. 89-days on vitamin A deficient diet. Atrophy and metaplasia of enamel organ. Columnar odontoblasts still present on labial side. On mesial and lateral sides are gaps in the dentine which is elsewhere thin and much folded. Note the large areas of proliferative response composed of cells resembling osteoblasts. $\times 46.2$.

FIG. 4. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. There is restoration of the enamel organ and heavy calcification of the dentine. The layer of recovering odontoblasts and newly deposited dentine (predentine) shows clearly on both sides, also folding and gaps of the dentine with gross distortion of the shape of the tooth. On the mesial side there are several groups of ameloblasts incorporated in folds of the dentine and further embedded by dentine formed during repair. $\times 46.2$.

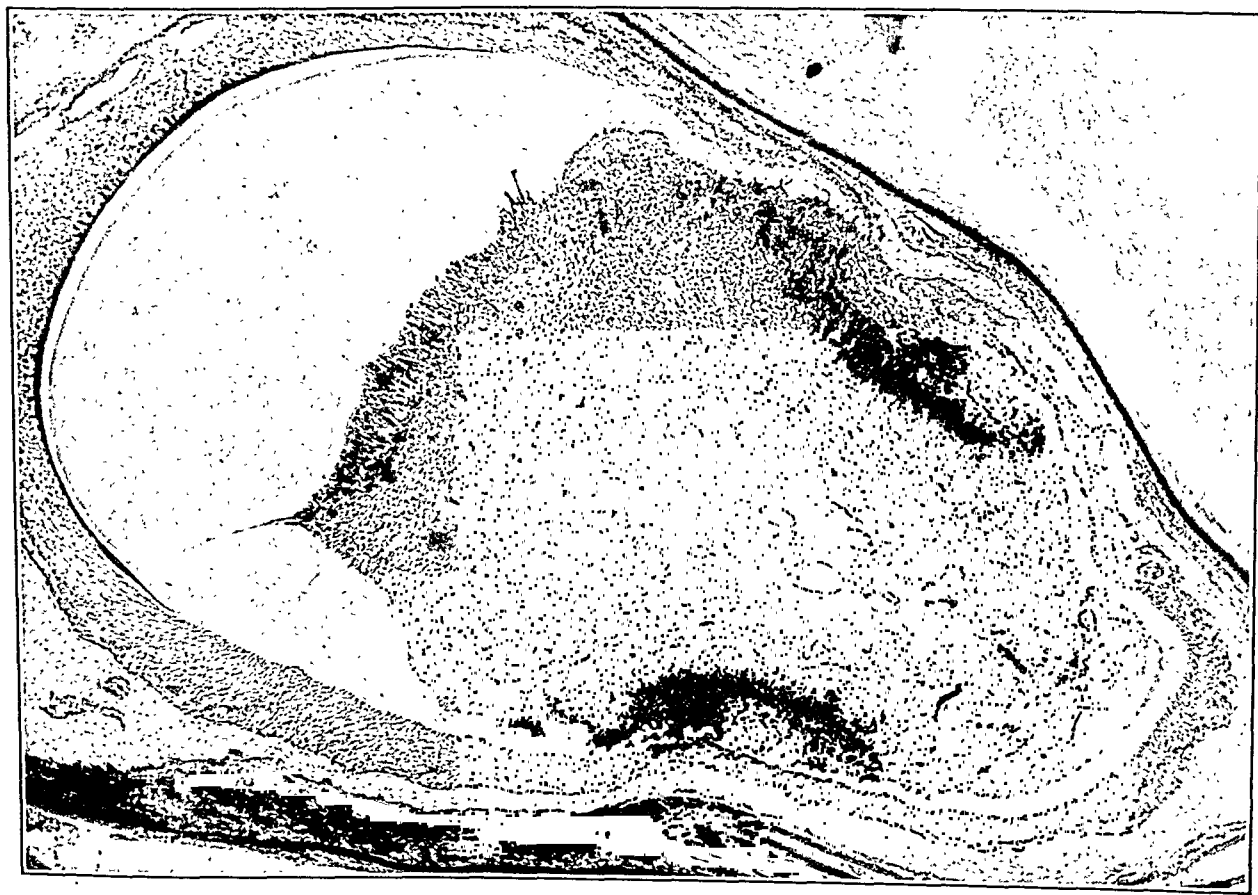


PLATE 40

FIG. 5. The enamel organ of rat fed upon the control diet in which butter fat replaced the lard of the deficient diet. $\times 588$.

FIG. 6. Rat 235. Upper incisor. 138 days on vitamin A deficient diet followed by 5 days with addition of butter fat. Shows extreme atrophy of enamel organ with globular deposits of calcified material in and adjacent to the atrophic remains of the epithelial papillae. $\times 588$.

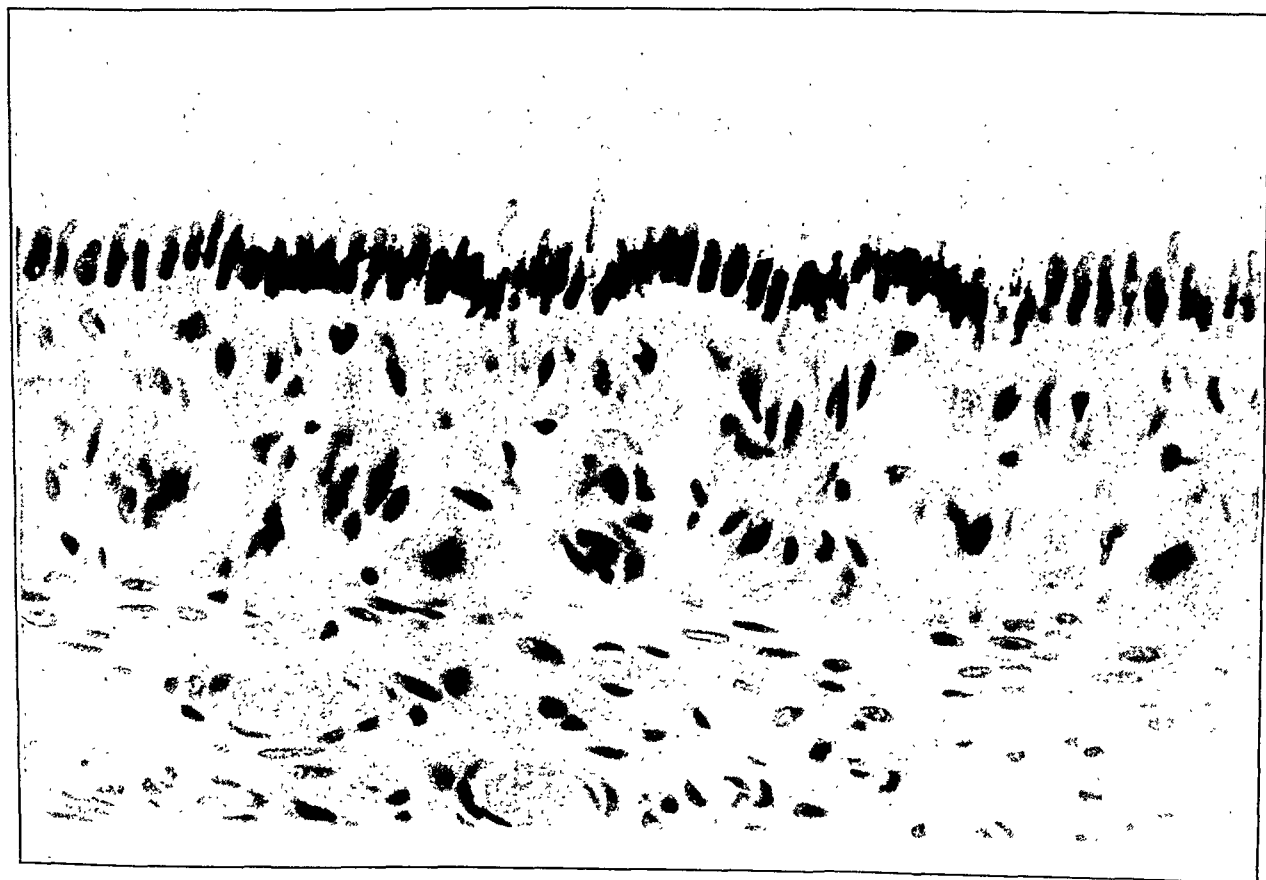


PLATE 41

- FIG. 7. Rat 412. Upper incisor at junction of mesial and labial sides. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. The preparation illustrates the differences in behavior of the odontoblasts of the labial side from those on mesial and lateral sides. The odontoblasts of the first show deposition of dentine under influence of restored diet. On the mesial wall the dentine is bounded by connective tissue-like cells, though early repair is shown in places by traces of dentine deposit between cells, some of which are arranged perpendicularly to the dentine. Note also nuclei of cells incorporated in the dentine. $\times 147$.
- FIG. 8. Rat 235. Upper incisor. 138 days on A deficient diet followed by 5 days with addition of butter fat. Shows typical shape of vitamin A deficiency rat incisor. Complete atrophy with metaplasia of the enamel organ. Complete atrophy of odontoblasts except on the labial side where the atrophy is nearly complete. Continued function of some cells has resulted in the incorporation of others by the most recently deposited dentine. $\times 46.2$.



PLATE 42

FIGS. 9 and 10. Rat 103. Sections of both upper incisors through formative ends. 180 days on vitamin A deficient diet. Fig. 9 shows particularly well the folding of the dentine which makes possible the inclusion of ameloblasts. Both photographs show clusters of proliferated odontoblasts and in Fig. 10 the gland-like formations due to ameloblasts free in the pulp at A. For high power see Figs. 11 and 12. $\times 46.2$.



PLATE 43

FIGS. 11 and 12. High power details of Fig. 10 showing inclusions of atrophied ameloblasts in the pulp. In Fig. 11 osteodentine is being formed by pulp cells external to the inclusions of ameloblasts. $\times 588$.

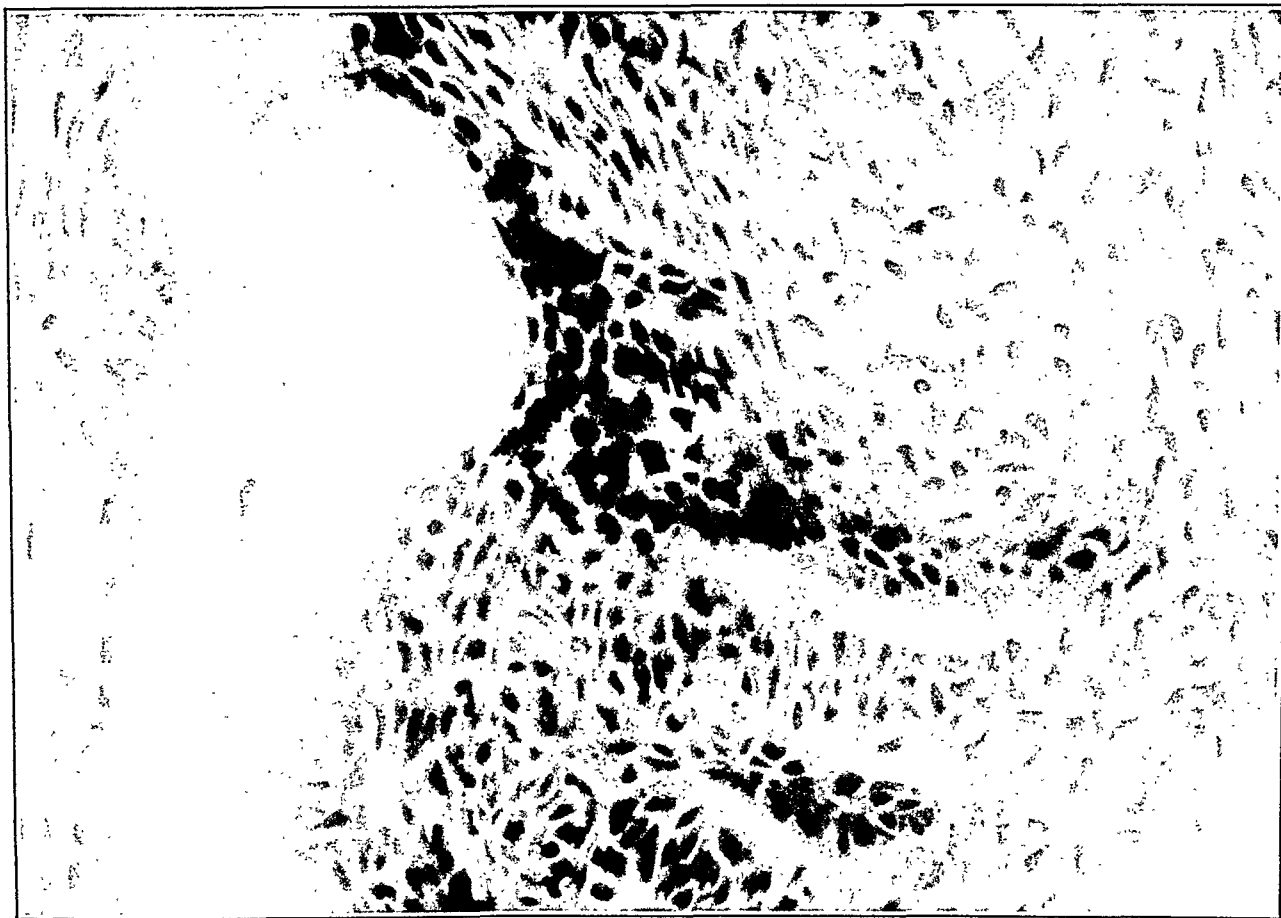


PLATE 44

FIG. 13. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. At A and B are ameloblast inclusions in the dentine. Newly formed dentine has filled spaces between the folds of dentine and has surrounded the inclusions of ameloblasts. For high power detail see Fig. 14. The restoration of odontoblasts is shown in both Figs. 13 and 14. Heavy calcification of the dentine on the labial side of the tooth is shown, a usual response to restoration of vitamin A. $\times 46.2$.

FIG. 14. Drawing. A detail from Fig. 13 at B. Shows dentine formation about ameloblast inclusion as a result of restoration of vitamin A to diet. The odontoblasts have not yet recovered normal size and form, yet much dentine has been deposited and calcified. The irregular outline of the folded dentine of the deficiency period is less deeply calcified than the dentine deposited during the recovery period. The ameloblast inclusion was probably not surrounded by dentine during the deficiency period. $\times 300$.

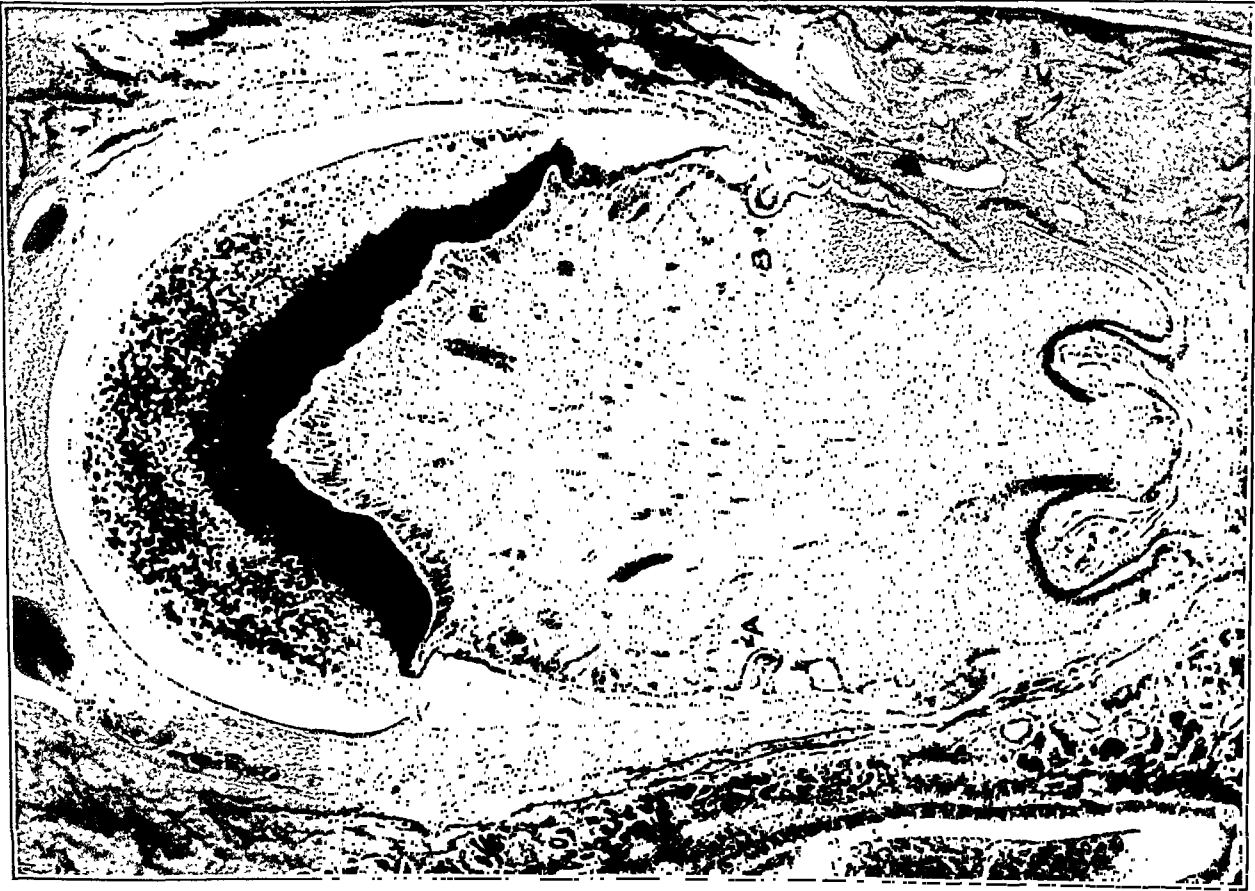
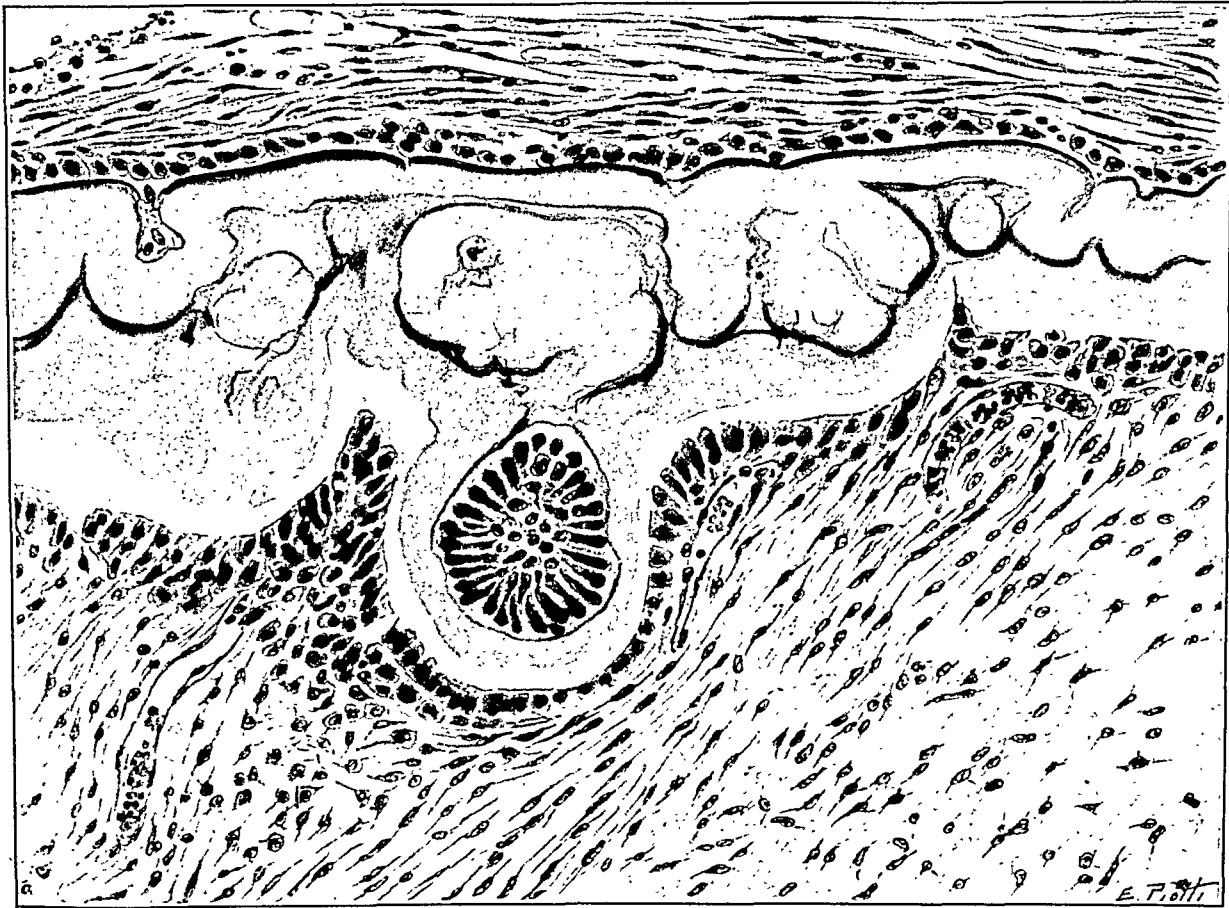


PLATE 45

FIG. 15. Rat 417. Upper incisor. 187 days on vitamin A deficient diet followed by 7 days with addition of butter fat. To show gland-like formation of ameloblast inclusions in pulp in recovery from atrophy. Note mitotic figure. $\times 588$.

FIG. 16. Rat 412. Lower incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. To show growth of denticles in consequence of restored diet. Heavy calcium deposits in old dentine. $\times 46.2$.

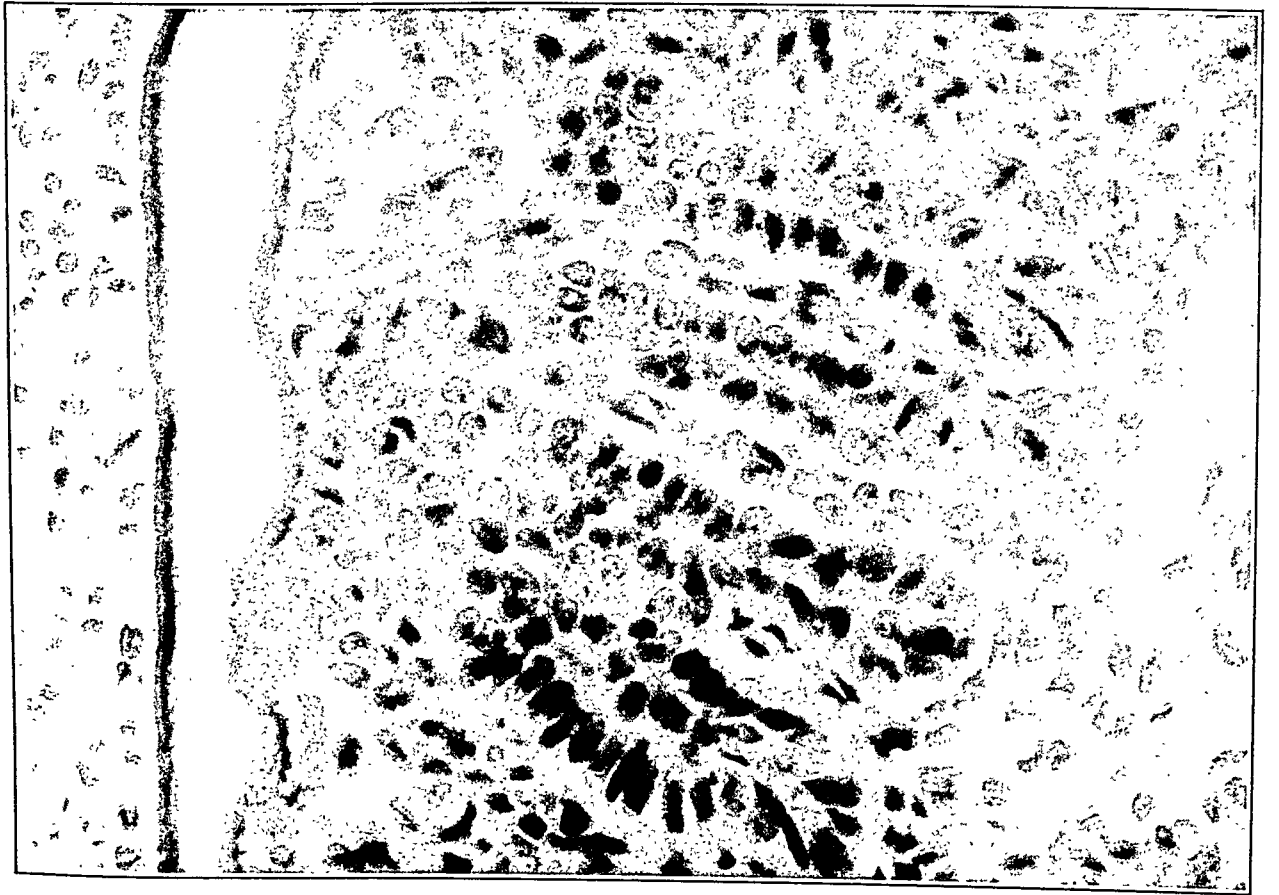


PLATE 46

FIG. 17. Rat 401. Upper incisor. 161 days on vitamin A deficient diet followed by 22 days with addition of butter fat. Denticles of both types showing large deposits of dentine formed since restoration of the diet. $\times 46.2$.

FIG. 18. High power detail of Fig. 17 showing that the odontoblasts, though functionally active, are not yet completely restored in morphology. Inclusions of ameloblasts have disappeared. $\times 147$.



PLATE 47

- FIG. 19. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. To show odontoblasts formed about ameloblast inclusions in the pulp. The former have deposited a considerable amount of dentine. The latter are atrophic. $\times 588$.
- FIG. 20. Guinea pig 142. 128 days on experimental diet without source of vitamin A other than 4 cc. of orange juice daily. Shows nearly complete atrophy and disappearance of enamel organ with calcified deposits in the atrophic remains of the enamel epithelium and external to it. $\times 294$.

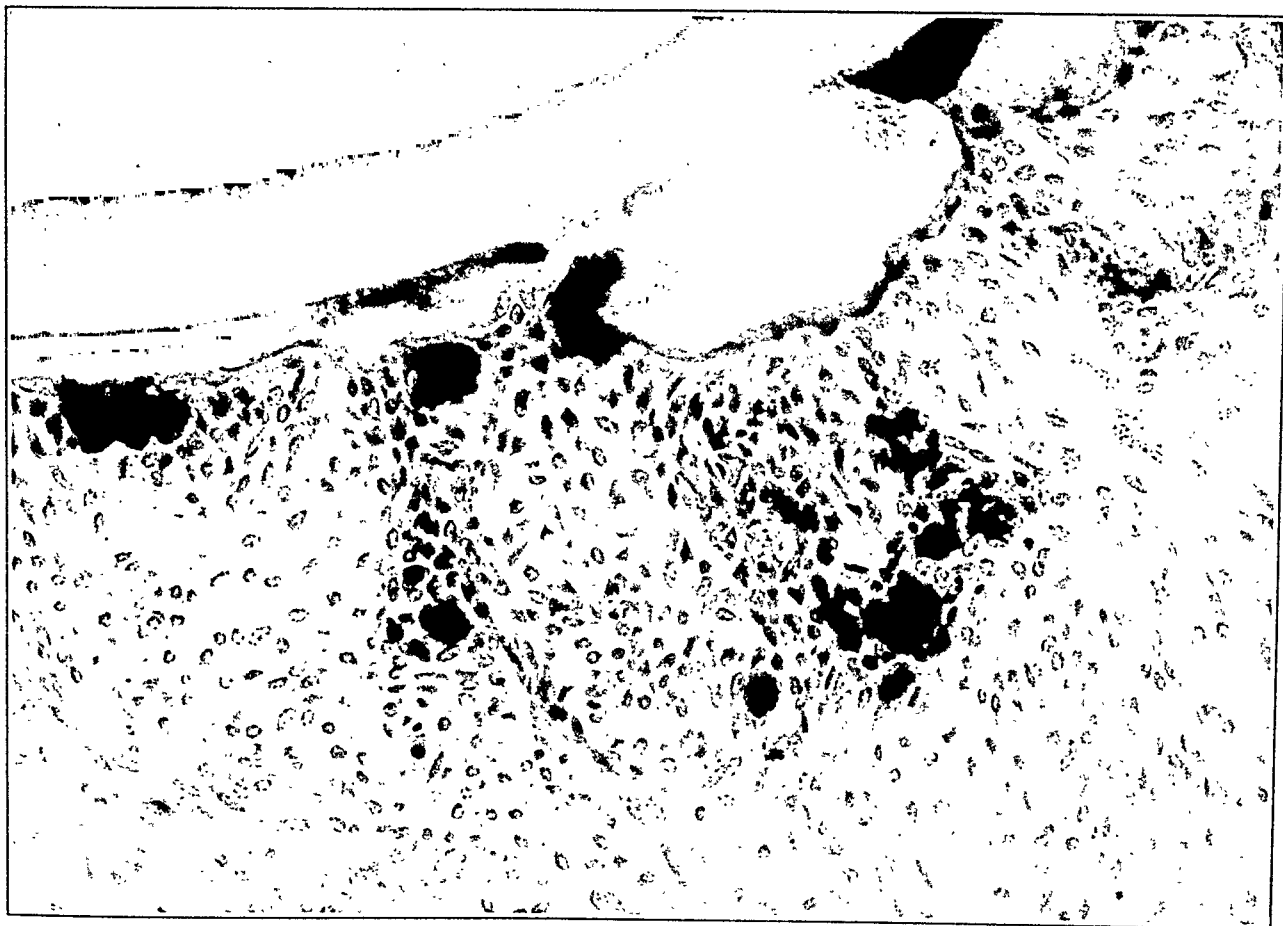


PLATE 48

FIG. 21. Guinea pig 142. Lower incisor. Extreme atrophy and calcification of enamel organ. $\times 294$.

FIG. 22. Guinea pig 38. 91 days on experimental diet without source of vitamin A other than orange juice, of which it received 20 cc. daily for 56 days, 8 cc. daily for 21 days and 4 cc. daily for 24 days. Ossification of enamel organ. $\times 147$.

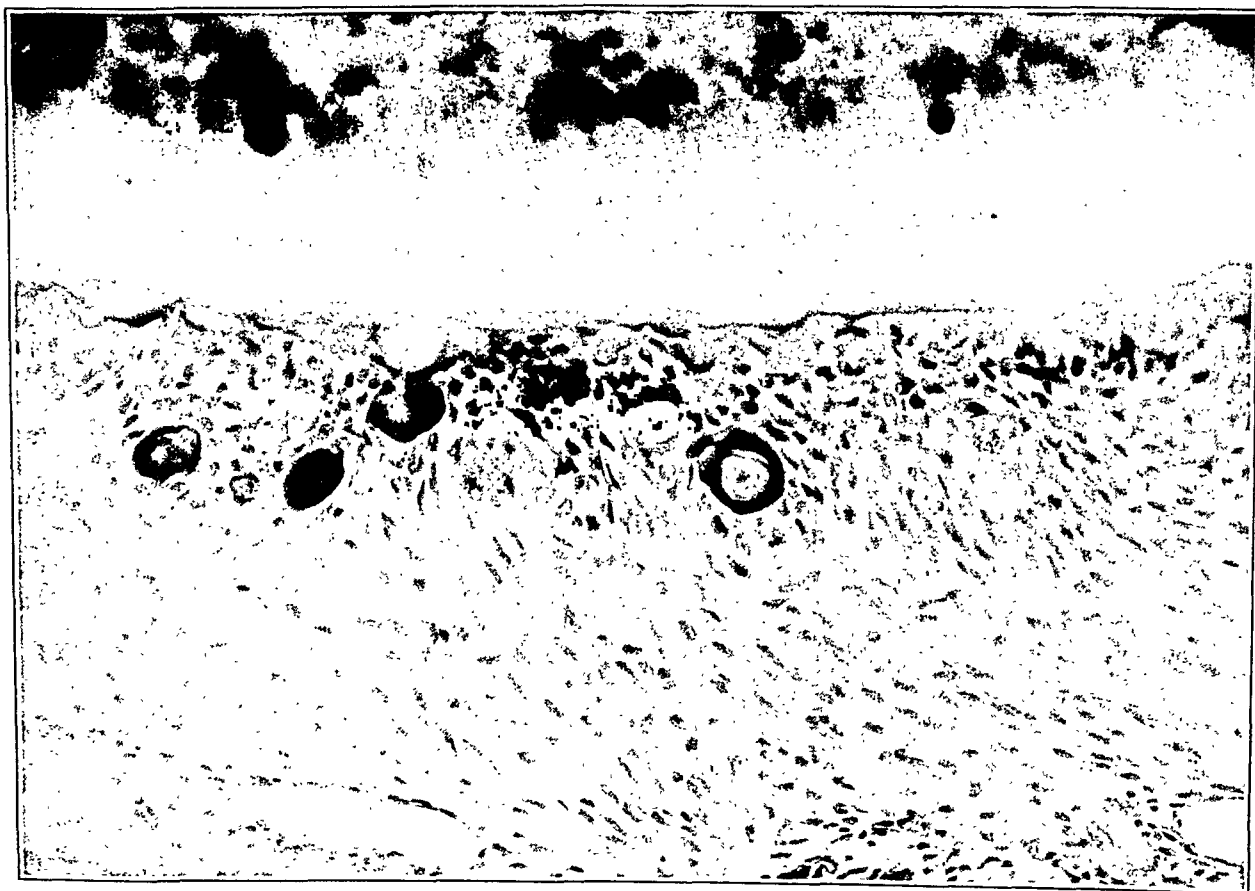
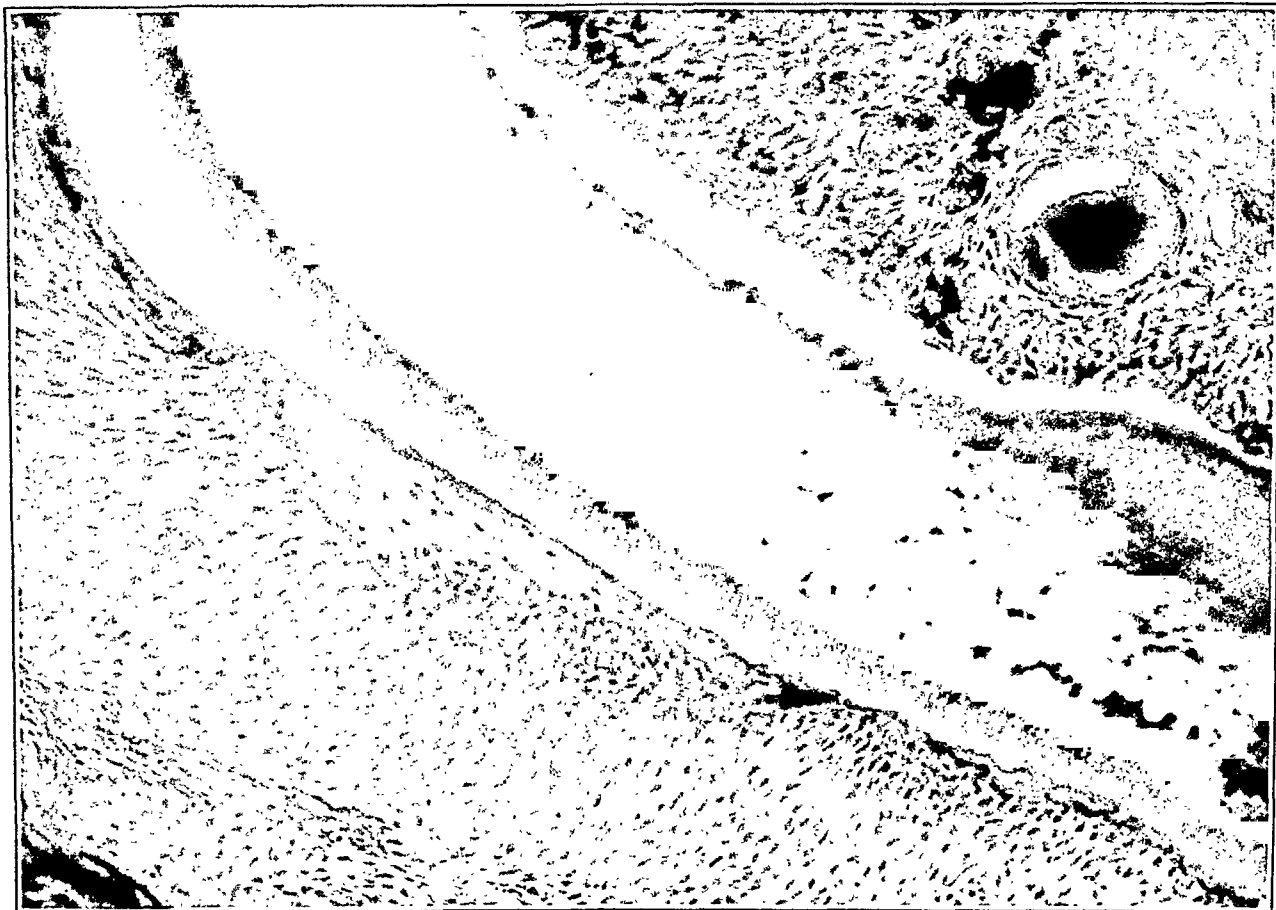


PLATE 49

- FIG. 23. Guinea pig 38. Same preparation as Fig. 22. Another region. At each end of the bony plaque and external to it, remains of the enamel epithelium can be seen. $\times 147$.
- FIG. 24. Guinea pig 38. Lower power. Same preparation used for Figs. 22 and 23. Shows ossification of the enamel organ, cementicles, and on the lingual side, numerous ingrowths of osteodentine. $\times 46.2$.

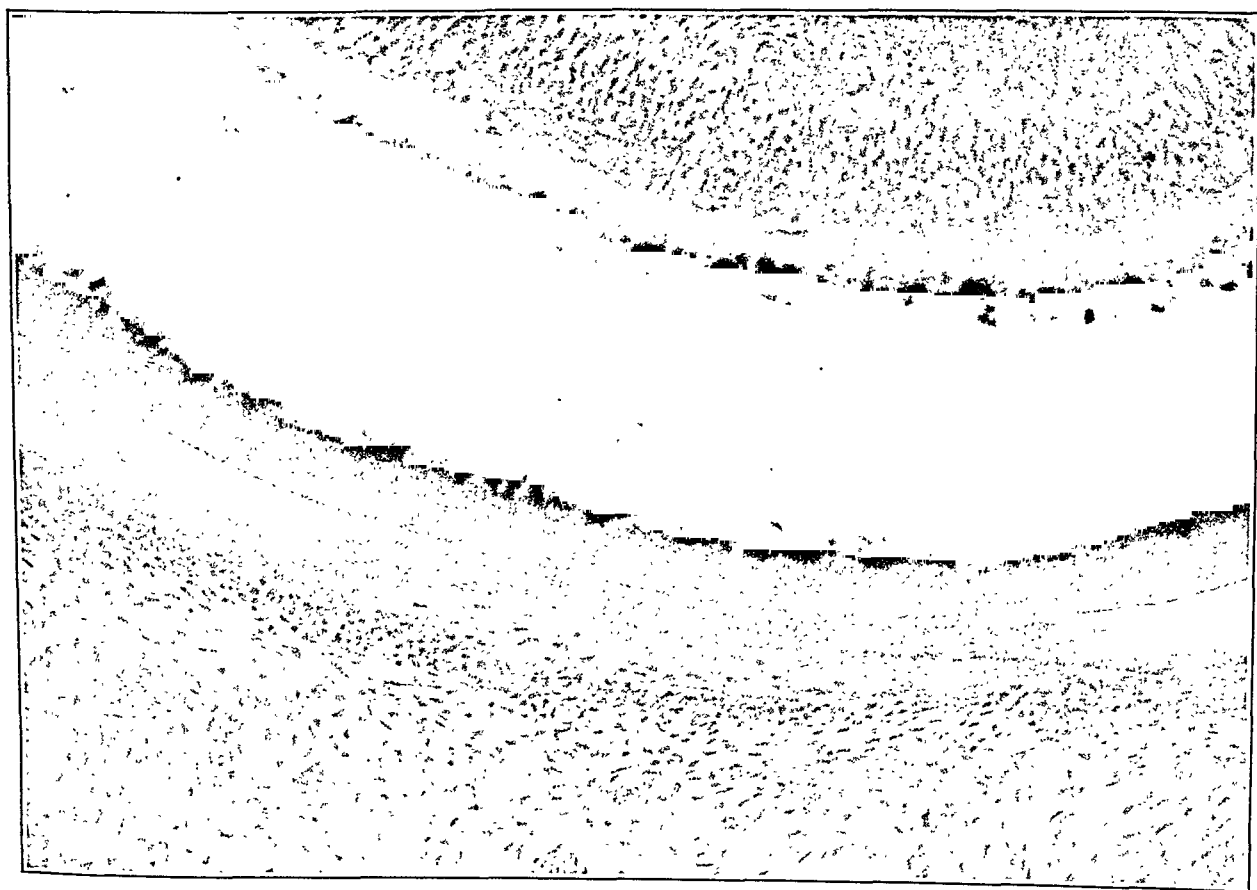
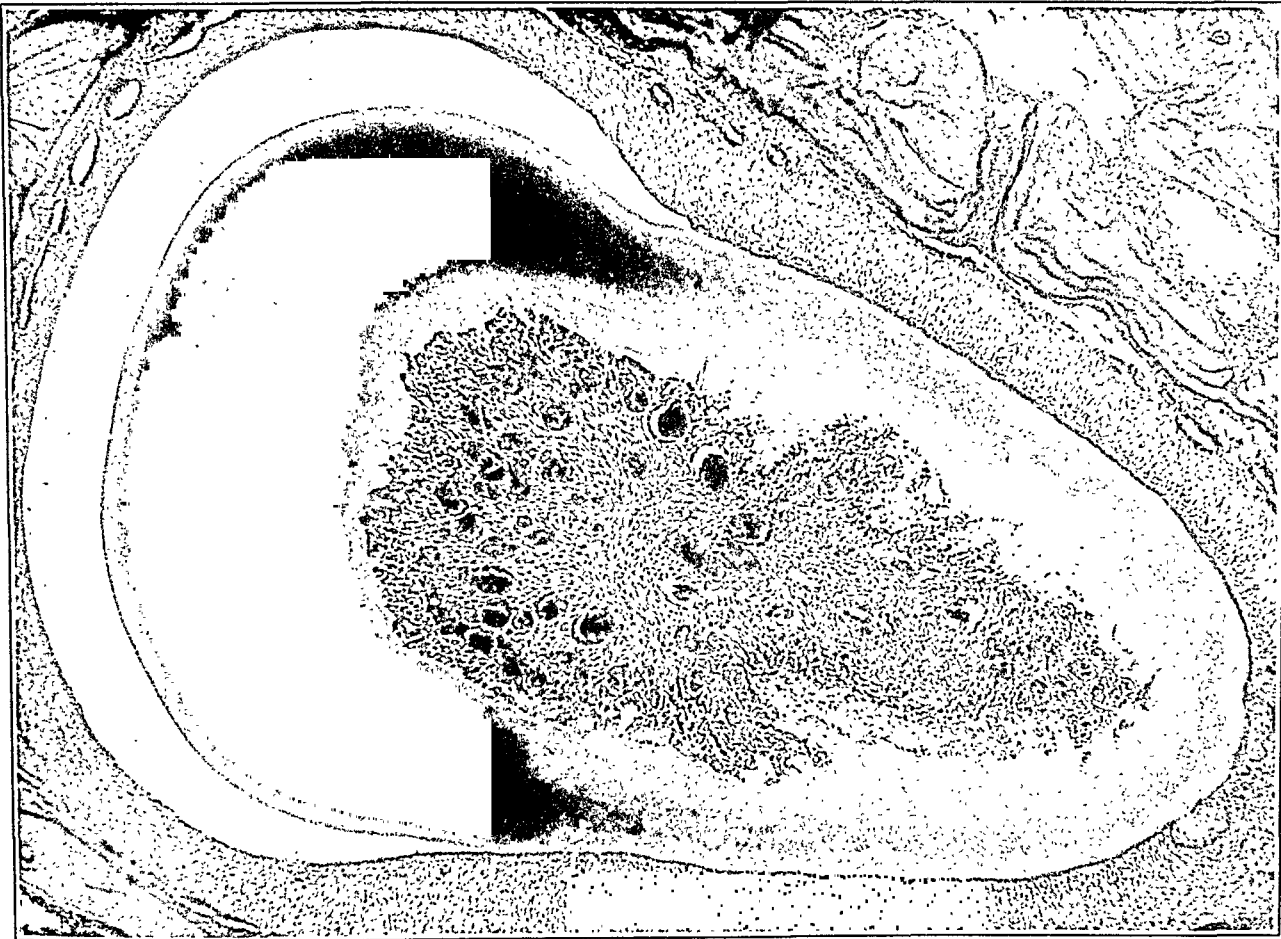


PLATE 50

FIG. 25. Guinea pig 41. Upper incisor. 299 days on experimental diet without source of vitamin A other than orange juice. For 207 days it received 20 cc. of orange juice daily, 10 cc. daily for 15 days and 5 cc. for the last 75 days. Shows nearly complete atrophy of enamel organ and of odontoblasts, except on labial side of tooth. In the guinea pig, in contrast to the rat, atrophying odontoblasts because of depolarization act like osteoblasts and deposit matrix centrifugally. The excrescences of osteodentine, or denticles, are of considerable size. $\times 46.2$.

FIG. 26. Guinea pig 46. 158 days on experimental diet without source of vitamin A other than orange juice, of which it received 20 cc. daily for 28 days and 8 cc. daily for 130 days. Note cementum outgrowths, cementicles and atrophy of odontoblasts with osteodentine formations. $\times 46.2$.



SPONTANEOUS RUPTURE OF THE HEART*

REPORT OF 40 CASES IN PORTLAND, OREGON

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REVIEW OF LITERATURE

The great William Harvey¹ in 1647, besides describing the circulation of the blood, recorded the earliest observation of cardiac rupture. His patient was a nobleman who often suffered with paroxysms and oppressive pains in the chest. Finally, "the disease growing more violent, he became cachectic and dropsical, and at length, being violently oppressed in one of the paroxysms, he died. In this patient, by reason of the circulation of the blood being obstructed from the left ventricle into the artery, the very substance of the left ventricle itself, which seemed to be pretty thick and strong, had been ruptured and perforated with a large opening, where it had discharged its blood; for the foramen was sufficiently large to admit a man's finger."

In 1765 Morgagni,² the eminent morbid anatomist and president of the University of Padua, collected 10 cases and added an observation of his own, "made upon a most powerful monarch." It is of a peculiar grim interest that Morgagni himself eventually suffered the same type of death.

Morand³ in 1732 who, Morgagni says, had "written and collected more than any other person ever did yet on the subject of rupture of the heart," was probably the first to associate this condition with myocardial softening. Then came the observations of Verbrugge, Portal and Laennec.⁴

In 1757 Galeati⁵ published the first account of an aneurysm of the left ventricle and described rupture of this into the pericardial sac.

Blaud⁶ in 1820, referred to by Cruveilhier as "that estimable observer, who, in a small country town has so often paid his tribute to science" collected 4 cases of rupture, and attributed them to a soft

* Received for publication December 27, 1932.

gelatinous degeneration of the myocardium. Likewise Fleury ⁷ in 1812, Rostan ⁷ in 1820, Dézeimeris ⁸ in 1839, and Cruveilhier ⁹ in 1849 made observations that at least contributed to the knowledge of this cardiac accident.

In Dublin arose a school of writers including such noted names as Cheyne ¹⁰ in 1818, Robert Adams ¹¹ in 1827, Townsend ¹² in 1832, Smith ¹³ in 1836, Stokes ¹⁴ in 1846, Bellingham ¹⁵ and Kennedy ¹⁶ in 1849, who designated the senile or gelatinous degeneration described by the French writers by a different name — fatty degeneration — and attributed cardiac rupture to this cause. Adams described a rupture of the left ventricle in a gentlewoman over 60 years old, of corpulent habit and “confined bowels” who was attacked with pain in the epigastrium. Fomentations and liniments on the abdomen, pills containing blue pill and calomel, then a further purgative draught of infusion and tincture of senna and Rochelle salts, then a terebinthinate enema followed by “bleeding,” all failed to relieve her and she died suddenly in a fit of vomiting. Autopsy showed rupture of the left ventricle, whose inside was so soft “as to admit of being broken down between the finger and thumb.” In England Williams ¹⁷ in 1840, and Quain ¹⁸ in 1850 followed the same lead but at least evinced the suspicion that coronary narrowing might be the underlying cause.

As early as 1815 one English author, Joseph Hodgson,¹⁹ expressed an opinion that comes close to the modern conception of coronary disease. He reported rupture of the left ventricle where “the degeneration of the structure of the ventricle had in one part proceeded to such an extent that the remaining fibers were unable to resist the force of the circulation.” He found the left coronary artery completely occluded by calcareous deposit, but like all others of that period overlooked thrombosis of the coronary arteries.

This early phase of coronary study ended with several noteworthy reviews. In 1857 Elléaume’s important Paris thesis ²⁰ collected about 50 spontaneous ruptures up to that time, and in 1871 Barth ²¹ added 24 more. Many causes had been assigned for heart rupture — most of them erroneously. To be sure several authors had described senile softening of the myocardium and a few, including Barth, had connected this with atheromata of the coronaries. Cruveilhier ²² had in 1850, according to Huchard,²³ even reported coronary thrombosis, without appreciating its full significance. But none had even hinted

at the now recognized sequence of coronary sclerosis — thrombosis, infarction and rupture.

The second period in the study of cardiac infarction and rupture starts probably in 1861 with Malmsten.²⁴ This Swedish observer was the first definitely to affirm a relationship between coronary thrombosis, myocardial softening and rupture. In describing a rupture of the left ventricle he says: "Microscopically the muscle fibers were here destroyed and replaced by a highly granular detritus, mingled here and there with fatty granules. In places there were small areas, visible even to the naked eye, where only connective tissue remained, buried in the degenerated mass." The anterior branch of the left coronary artery contained an old white thrombus situated just before the entrance of the artery into the degenerated area. "The clot in the coronary artery was undoubtedly old. The arterial obstruction had resulted in a slow softening of the muscular tissue. Finally rupture appears to have occurred slowly." It seems only fair to regard the foregoing as a valid presentation of the concept of infarction.

In 1878 Ménard,²⁵ a Frenchman, definitely associated coronary thrombosis and rupture of the heart. In 1880 a physician in Boston named Winsor²⁶ made an epochal contribution. In describing a case of angina pectoris with rupture he reports: "In the vicinity of the rent — the characteristic appearance of the muscle was lost, the muscular fibres being here filled with a granular material, and in many places with minute fat drops." The walls of the left coronary artery were thickened and sclerotic and their lining covered with yellowish white patches. "At one point, about 3 cm. from the origin of the artery, one of these patches had so far protruded into the lumen of the vessel as to cause a coagulation of blood at that point, which coagulation had become adherent to the walls, thereby preventing the flow of blood through the vessel. The portion of the heart in which the rupture had occurred and in which the fibres were found degenerated corresponded to the territory supplied by the branches of this artery." This constitutes probably the first entirely valid presentation of the etiology of heart rupture.

Other corroborative reports appeared about this time. In Germany Weigert²⁷ in 1880 enunciated the principle of cardiac infarction and was followed by Ziegler,²⁸ Cohnheim and von Schulthess-Rechburg,²⁹ and Huber.³⁰ The interrelation of coronary thrombosis, myocardial ischionecrosis and rupture is further supported by the

writings of Samuelson ³¹ in 1881, Birch-Hirschfeld ³² in 1883, Leyden ³³ in 1884, and Georg Meyer ³⁴ in 1888 in Germany; Poppoff ³⁵ in 1882 in Russia; Mollière and Duplaix ³⁵ in 1883 in France; and in England of Steven ³⁶ in 1887, who mentions other lesser contributions of that time.

René Marie ³⁷ in his noted Paris thesis of 1896 sums up the results of these various writers and concludes that the lesion that determines rupture is not, as Odriozola ³⁸ in 1888 had maintained, a progressive arteriocardiac ischemia, but infarction from sudden thrombosis of an artery.

Since René Marie's time little further progress has been made in the development of the morbid anatomy and pathogenesis of heart rupture. Thrombosis and infarction are accepted as the inciting cause. At present the question arises if we may not now be embarking on a third phase of the problem. Experimentation is being directed toward a solution of the underlying causes of arteriosclerosis, thrombosis and infarction. Also, since there are other sources of heart rupture besides infarction, it is essential to differentiate these in each case.

INCIDENCE OF CARDIAC RUPTURE

Cardiac rupture appears never to have been reported up to the time of Harvey's publication of his case in 1647.¹ Probably only 10 instances appeared during the following century, according to Morgagni.² In the course of still another century, Elléaume ²⁰ was able to add 47 more. After this the number increased rapidly. Barth ²¹ in 1871 collected 24 from recent reports in the *Bulletin de la Société d'Anatomie* from 1826 to 1865 and Odriozola ³⁸ in 1888 added 110 more.

The latest tabulations indicating a greatly increased incidence are those of Krumbhaar and Crowell ³⁹ in 1925, who collected from the literature (excluding Elléaume and Barth) 632 ruptures, exclusive of 22 unpublished cases from Philadelphia hospitals, and Davenport ⁴⁰ in 1928, whose supplementary list of 92 further instances raised the total to 734. This number, while high as compared with previous figures, does not represent an incidence nearly as great as is found in some autopsy services and probably includes but a small fraction of the real total.

The percentage of cardiac ruptures in hospital morgues is always

low, for the obvious reason that such patients are usually not spared long enough to reach a hospital. It will suffice if we cite Krumbhaar and Crowell's ³⁹ figure of 7 ruptures in 16,000 autopsies at the Philadelphia General Hospital; the Munich report of 7 in 13,000, as cited by Georg Meyer ³⁴; and the Leipzig series of 9 in 8000 (Krumbhaar). De la Chapelle ⁴¹ has more recently recorded 20 cases of rupture among 15,000 autopsies in the medical examiner's service in New York City. A notable exception among hospital statistics is the incidence of 0.86 per cent of cardiac ruptures among 2374 autopsies, reported by Beresford and Earl ⁴² in 1930 from the Tooting Bec Hospital in England, an institution whose patients are largely senile persons suffering from mental disorders. To Beresford and Earl also belongs the credit for reporting the largest number of cardiac ruptures thus far studied by a single group.

Our figure of 40 ruptures of the heart among nearly 7000 autopsies (0.57 per cent) is in sharp contrast to all figures heretofore obtained, with the exception of Beresford and Earl's. ⁴² Its explanation admits no doubt. Most of these patients died without medical attention, 27 reaching the coroner's morgue. Of the remaining 13 examples 3 were from private practice and 10 died in the hospital. Werley, ⁴³ in a smaller series, reports an incidence of cardiac rupture equal to that of our coroner's group. He encountered 3 ruptures among 200 autopsies in El Paso during two years (1.5 per cent).

ETIOLOGY AND CLASSIFICATION

There was a period when it was considered ridiculous to hold that cardiac rupture could be caused by any other agent than fatty degeneration of the heart. Thus Quain ¹⁸ deplored the fact that Corvisart and Cruveilhier doubted the rôle of fatty change in producing myocardial softening.

The research of a century has now demonstrated the importance of coronary arterial disease as the source of heart rupture. Most authors, perhaps too freely, admit that the only factor worthy of consideration is coronary occlusion. They assume, often without ample histological verification, that the sequence of events is invariably as René Marie ³⁷ maintained in 1896, — coronary sclerosis,

thrombosis, infarction	{	rupture,
		aneurysm,
		myofibrosis.

They are probably quite correct in maintaining that this order usually holds, but a suspicion arises that they are on occasion, by blindly following the accepted doctrine of the preceding age, missing an opportunity to demonstrate other causes of rupture.

It is reasonable to suppose that rupture may be induced by any occlusion of coronary arteries, especially recent occlusions, with or without anatomical evidence of infarction, but it is equally plausible that the heart wall may break if its resistance is lowered by disease of the myocardium independently of disease of the nutrient arteries. It is also possible to find myocardial changes, such as fatty infiltration or fatty degeneration, coincident with arterial obstruction.

We shall therefore attempt to classify cardiac rupture, first as to site and then according to the various causes found in the more authentic reports.

Rupture may occur:

- (a) Through the ventricular wall externally.
- (b) Through the auricular wall externally.
- (c) Through a portion of the thickness of the external wall, by coronary rupture and dissecting hemorrhage.
- (d) Interventricularly or interatrially through the septum.
- (e) Through the heart wall internally or externally by a dissecting aneurysm of the aortic base.
- (f) In papillary muscles.
- (g) In valve cusps.

Cardiac ruptures usually occur in the left ventricle, less often the other chambers, at the site of recent infarction or occasionally in old cicatrized areas with fresh necrosis. In Davenport's ⁴⁰ tabulation the left ventricle was the seat of rhexis in 566 (79.8 per cent); the right ventricle in 76 (10.7 per cent); the right auricle in 38 (5.3 per cent); and the left auricle in 13 (1.8 per cent). Ruptures of the anterior left ventricular wall predominated with a ratio of 3 to 1 over the posterior surface.

Other seats of rupture are: The papillary muscles, 9 instances in all — Corvisart ⁴⁴ 1812; Bertin ⁴⁵ 1824; Dennig ⁴⁶ 1909; Wankel ⁴⁷ and Teacher ⁴⁸ 1911; Fischer ⁴⁹ 1922; VonGlahn and Horowitz ⁵⁰ 1923 (coronary thrombosis case); Eichhorst ⁵¹ 1921, the last being reported as due to syphilis (as was also the case reported by Spalding and VonGlahn ⁵² the same year, with demonstration of spirochetes); the interventricular septum, usually, though not always, in the pars

membranacea — see Markham⁵³ 1859, Pergami⁵⁴ 1876, Crowther⁵⁵ 1880, Meyer³⁴ 1888, Youmans⁵⁶ 1921, Faulkner, Marble and White⁵⁷ 1924, Martin and Waugh⁵⁸ 1925, Werley⁴³ 1925, Hyman⁵⁹ 1930, Nickson and Benson* 1931 and our 2 cases reported here, about 26 in the literature; and the valves, represented by Howard⁶⁰ and Thayer⁶¹ 1925. Many more might also be cited.

Dissecting aneurysms are made the subject of a detailed thesis with long bibliography by Vestberg⁶² in 1897, who collected 59 cases besides his own single one. He classifies them as parietal, septal, valvular, and interparietal, the point of predilection being, however, at the junction of heart and aorta. From this point of origin the hemorrhage may dissect through the heart wall and rupture into the pericardial sac or into an auricle. We have had one which, starting in a sinus of Valsalva, dissected through the interauricular septum and bulged into the left auricle without rupturing. Our other aneurysm started similarly, bulged into the right auricle, but finally ruptured anteriorly into the pericardial sac. Claytor⁶³ had a similar case.

The coronary arteries themselves may rupture into the myocardium, and thence usually into the pericardial sac, as in the reports of Galliardi⁶⁴ 1840, Odriozola's³⁸ 5 examples in 1888, Dock,⁶⁵ and Rochester⁶⁶ 1896, LeCount⁶⁷ 1918, Kalyanvala⁶⁸ 1919, Kesteven and Verco⁶⁹ 1920, Jones-Evans⁷⁰ 1922, Benson and Hunter⁷¹ 1925, and Lewis⁷² 1925. Coronary aneurysm is reported by Odriozola,³⁸ LeCount⁶⁷ and others.

The pathogenesis of rupture, according to the best reports available, may be classified as follows:

1. Cardiac infarction due to thrombosis or embolism is admittedly the commonest cause and appears throughout the modern writings. Arteriosclerotic disease of the coronary arteries is the basis of most thromboses, while embolism may arise from distant pyemic abscesses, from endocarditic vegetations (Huber³⁰ 1882, LeCount⁶⁷ 1918); from floating thrombi, as reported by numerous authors, or from atheromatous plaques loosened off from the lining of an artery and carried along in the circulation. Of this latter type Panum,⁷³ in 1862, reported the well known case of Thorwaldsen, who died suddenly in a Copenhagen theater. Dock⁶⁵ 1896, and LeCount⁶⁷ 1918, reported similar cases and we are reporting another. Allbutt⁷⁴ 1921, also discusses accidents of this kind in syphilitic arteries.

* Unpublished case.

2. Cardiac aneurysm is occasionally the seat of rupture, but usually only in instances in which a fresh coronary occlusion supervenes. We are reporting some cases of this type.

3. Gummas with rupture were, according to Georg Meyer³⁴ first described by Oppolzer, but they also have nearly disappeared from the literature as a cause. Diffuse syphilitic myocarditis, on the other hand, is occasionally blamed, probably in certain instances with truth, as in the three examples of Harlow Brooks⁷⁵ in 1921. Aubertin⁷⁶ in 1924, who gives a good summary of the pathology of rupture, cites exceptional instances of rupture without localizing lesions, but with chronic myocarditis or fatty degeneration of the myocardium. It would seem that such cases might well be stained for spirochetes. In this connection the reader is referred to Warthin⁷⁷ 1918, 1925.

4. Authentic reports of rupture of tuberculous lesions are represented by Korybut-Daszkiewicz⁷⁸ 1922, Oudendal⁷⁹ 1923 and Brandi⁸⁰ 1931. Tuberculosis of the myocardium is occasionally reported in cases without rupture. Of special interest are two reports by Swiss investigators, Massini⁸¹ and Lüscher⁸² in 1921, of masked tuberculous myocarditis with animal verification.

5. Endocarditis appears frequently in the older writings as a cause of rupture, as in Böttger's 3 cases, cited by Meyer,³⁴ but is usually to be discounted. The more reliable recent reports include those of Claytor⁶³ in 1923, Locke⁸³ in 1925, and a few others. Claytor's appears to be in reality a dissecting aneurysm with rupture and we are including a similar case in our series due to *Streptococcus viridans*. Nickson and Benson* have also observed an instance of perforation of the membranous interventricular septum due to erosion by subacute *Streptococcus viridans* endocarditis of the tricuspid valve. Endocarditis as a direct cause of rupture is often open to doubt unless minute examination of the coronary arteries for embolism has been made.

6. Meyer³⁴ in 1888 cited what he thought was the only then known case of rupture of the heart due to echinococcus cysts, that of Dézeimeris⁸ in 1834, but he evidently overlooked the report of Crowther,⁵⁵ who in 1880 described a perforation of the interventricular septum by rupture of a hydatid cyst. Echinococcus of the heart without rupture has also been reported; thus Hynd⁸⁴ in 1924, in a case of sudden collapse and death with respiratory failure, found that

* Unpublished case.

in the right ventricle a subendocardial cyst the size of a hen's egg had emptied its contents, consisting of smaller cysts, which proved microscopically to be echinococcal. Several cysts were found in the branches of the pulmonary arteries.

7. Malignancy is sometimes mentioned as a cause of rupture, but Meyer³⁴ failed to find any ruptured neoplasms in the reports up to 1888, and Jores⁸⁵ in 1924 discovered none in the recent literature. Krumbhaar and Crowell,³⁹ however, include a melanotic sarcoma in their tabulation, although they do not print the reference. Weiss⁸⁶ in 1880 also reported a large round cell sarcoma of the right auricle which had broken through the interauricular septum and produced metastases in the lung and other foci.

8. In a general treatise such as this, attention should be paid to the fact that cardiac rupture is not confined to the human species. Thus Meyer³⁴ says that it occurs in horses and cattle, and that Virchow and Hirsch's *Jahresberichten* reports 6 cases in animals in 10 years. Lewis⁷² also writes on rupture in animals.

Besides the various inciting causes listed above we should mention certain predisposing factors. Hippocrates, Celsus and Harvey referred to the frequency of sudden death in obese individuals, and many writers, beginning with Morgagni, have observed that rupture also occurred with high incidence in the obese.

Likewise adipose deposits in the epicardium have been noted by many as a predisposing cause. Fatty degeneration is, on the other hand, open to doubt as a causative factor, being probably rather an incidental by-product of infarction or, as Warthin⁷⁷ has pointed out, of syphilitic myocarditis. In fact Master⁸⁷ has shown the presence of fatty granules in normal heart muscle cells. The full stomach is claimed by various observers as a common predisposing factor in cardiorrhesis.

Silverthorn⁸⁸ mentions that rupture may occur in the absence of exercise, even during sleep, but Krumbhaar and Crowell³⁹ cite MacWilliams as authority for the statement that sleep is not always free from heart strain, and that during dreams or restless sleep the blood pressure may be increased and changes of rhythm such as heart block may appear. In most cases rupture follows some form of physical exertion.

When all these factors are properly evaluated there is one which stands out above all others, namely, coronary occlusion, with or

without anatomical evidence of infarction. The obstruction of arteries may be a slow one from sclerotic closure of coronary orifices, atheromata, arteriosclerosis, syphilitic arteritis, various chronic endarteritides; from pressure from without, notably in encasement by myocardial or other scars and in encroachment of dissecting aneurysms. Sudden obstruction of arteries, which is more important, is due to thrombosis, less often embolism; rarely dissecting coronary aneurysm; acute arteritis and endarteritis, and, as cited by Herri⁸⁹ in 1912, has even been caused by surgical tying-off of a coronary artery. This last was followed by infarct but not rupture. The commonest of all is a mixed type, the chronic arteriosclerotic narrowing complicated by sudden thrombosis or even embolism.

CASE REPORTS

The present report embraces 40 ruptures of the heart, of which 2 are perforations of the interventricular septum, 3 are ruptures of coronary arteries into the pericardial sac, and another an anterior cardiac rupture of a dissecting aneurysm of a sinus of Valsalva. We include, in distinction to de la Chapelle,⁴¹ all breaks in the continuity of any portion of the heart, the base line of the aortic valve being the upper boundary of what may be termed the heart. We, therefore, include ruptures, such as interventricular, which do not result in extravasation of blood into the pericardial sac. We have not, however, burdened our series with erosions of the chordae tendineae resulting from endocarditis.

Of these 40 ruptures, 35 have been encountered among a total of 5896 autopsies performed by the pathology staff of the University of Oregon Medical School, 2112 of which were done for the coroner of Multnomah County, the remaining 3784 private and hospital cases. Among the 35 ruptures in the Medical School group 27 were on the coroner's service. Of the remaining 13 private cases 10 died in hospitals, 5 of these being encountered among 873 autopsies performed by one of us (C. H. M.) at the Good Samaritan Hospital. This gives an incidence of 1.27 per cent cardiac ruptures in our coroner's series and 0.28 per cent in our private cases. For permission to publish Case 37 we are indebted to the U. S. Veterans' Bureau, a more extensive report of which has already appeared.⁹⁰ In a previous preliminary tabulation of coronary disease,⁷¹ we have already briefly mentioned

Cases 1 to 7, also 6, 7, 8, 30, 32, 33, 35 (Table I). In classifying our 40 cardiac ruptures as to the underlying morbid anatomy we find that they fall into several groups.

Group A. Rupture of Heart Due to Coronary Thrombosis (25 Examples)

Group A includes 25 instances of rupture of the heart through recent infarcts due to coronary thrombosis. Nearly two-thirds of all our cases fall in this category, a not surprising finding since the literature on cardiorrhesis is replete with examples of the type included in this class.

We shall therefore make only brief mention of our cases of rupture through acute infarcts and give more attention to the less common forms. The essential pathological data of Group A are listed in Table I. Seventeen of the 25 cases came to coroner's autopsy, 3 were private cases and 5 died in the hospital. In 16 instances death occurred suddenly and without forewarning, 9 others had a history of pain or other symptoms referable to a previous coronary attack. In 5 cases death came during or shortly following physical exertion: Case 2 after playing golf, Case 4 at the completion of his train run, Case 8 while working in the kitchen, Case 11 while shovelling snow, and Case 14 while painting a sign.

As shown in Table 1 there was in each of the 25 instances of rupture of recent infarct, except in Case 11 where the record was lost, a thrombus in the coronary artery supplying the area of rupture. Even in Cases 5, 10, 12 and 17, all of which appear to be syphilitic, coronary thrombosis and infarction were found. There seems to be no question then that these 25 examples constitute classical coronary thrombosis resulting in cardiac infarction and rupture. In this group approximately two-thirds were sudden deaths in the first coronary accident without previous symptoms referable to coronary occlusion.

Group B. Recent Infarct Superimposed on Old Cardiac Aneurysm (4 Examples)

CASE 26: (Path. No. 2225). Mrs. C. B., aged 65, a housewife, called her physician 17 days preceding her death, and complained of what she thought was la grippe, with aching, general malaise, and slight dyspnea and cough. The temperature at onset was 100° F, rising a few days later to 101°. In the course of her fatal illness precordial pain developed, with smothery feeling. Death came suddenly and unexpectedly, the only noteworthy sign being the terminal gasp.

Autopsy: This record was lost, but the heart was preserved. The apical portion of the left ventricle is thinned out to form an aneurysm, and gross evidence of fresh infarction is present in this, with dissecting hemorrhage and resulting rupture at the apex.

Histology: The microscopic picture is that of healed aneurysm with evidence of fresh infarction, especially in the margin of the aneurysm. Microscopic examination of sections of the coronary arteries discloses a thrombus with early organization in a branch of the left marginal artery.

Pathological Diagnoses: Rupture of recent infarct of left ventricle at site of old cardiac aneurysm; fresh thrombosis of branch of left marginal artery.

CASE 27: (Path. No. 9042). O. S., aged 71, housewife, died suddenly without medical attention and was subjected to a coroner's autopsy. No history was obtained.

TABLE I
Ruptures of Heart

Group	Case No.	Pathology No.	Sex	Age	Occupation	Weight of heart	Rupture	Cardiac lesion	Coronary occlusion			Myocardium				Gall-stones	Obesity
									Fresh thrombus	Organized thrombus	Coronary syphilis	Fatty infiltration	Fragmentation	Syphilis			
A	1	2137	M	37½	270 gm.	Left ventricle	Recent infarct	A.I.V.								
	2	5747	M	50+	Business man	400	"	"	A.I.V.			Both ventricles				+	
	3	6961	M	81	Laborer	500	"	"	L. circumflex			"	+			±	
	4	9126	M	68	Conductor	480	Coronary artery and left ventricle	"	"	"		"				+	
	5	9251	M	50	Quarryman	300	Left ventricle	"	A.I.V.		+					+	
	6	9879	M	65	Farmer	370	"	"	A.I.V.			L. ventricle	+		+		
	7	11209	M	86	Retired	450	"	"	L. circumflex								
	8	12981	F	60	Housewife		"	"	R. coronary A.			Both ventricles			+	+	
	9	15393	M	75	Gardener	350	"	"	R. coronary A.	A.I.V. and R. coronary A.	±	"	+				
	10	15636	M	54	Photographer		"	"	A.I.V. branch		+			+			
	11	15839	M	68	Candymaker		"	"	*	*	*	*	*	*	*	*	
	12	16362	M	75?	Canner	580	Coronary artery and left ventricle	"	"	A.I.V.**		+		+			

13	18785	F	73	Housewife	400	Left ventricle	"	"	A.I.V.		±			+	+
14	19013	M	77	Painter	275	"	"	"	A.I.V.	A.I.V.	±				+
15	20030	F	75	Housewife	400	"	"	"	L. circumflex						
16	20805	M	70	Laborer	440	"	"	"	A.I.V.						
17	21004	M	62	Laborer	430	"	"	"	L. circumflex		±***				+
18	22-1-30	F	79	Housewife	360	"	"	"	A.I.V. branch	A.I.V. branch				+	
19	132-3-30	M	65	Theater manager		"	"	"	L. circumflex						
20	178-4-31	M	60	Unknown		"	"	"	"	L. circumflex					±
21	428 GSH	M	60	Physician	450	"	"	"	R. coronary A.	A.I.V.					+
22	706 GSH	M	52	Salesman		"	"	"	L. circumflex						+
23	846 GSH	F	56	Housewife	300	"	"	"	R. coronary A. L. circumflex						
24	307-7-32	M	69	Truck driver	470	Left and right ventricles	"	"	R. coronary A.	A.I.V.					±
25	317-7-32	M	78	Retired	550	Left ventricle	"	"	A.I.V.						
26	2225	F	65	Housewife		"	"	Aneurysm and infarct	L. marginal						
27	9042	F	71	Housewife	380	"	"	"	"	A.I.V.		+			+

B

A.I.V. = Anterior interventricular branch of left coronary artery.

L. coronary A. = Left coronary artery.

L. circumflex = Left circumflex coronary artery.

L. marginal = Left marginal coronary artery.

R. coronary A. = Right coronary artery.

* Heart lost before description completed.

** Thrombosis of A.I.V. and branches with dissecting hemorrhage of these vessels.

*** Syphilis of aorta.

TABLE I (continued)

Group	Case No.	Pathology No.	Sex	Age	Occupation	Weight of heart gm.	Rupture	Cardiac lesion	Coronary occlusion			Myocardium			Gall-stones	Obesity
									Fresh thrombus	Organized thrombus	Coronary syphilis	Fatty infiltration	Fragmen-tation	Syphilis		
B	28	11716	F	65	Housewife		Left ventricle	Aneurysm and infarct	A.I.V.	A.I.V.				+	#	
	29	13543	M	56	Physician		" "	" "	A.I.V.					+	#	
C	30	10160	M	76	Watchman	390	" "	Septic infarct	Multiple emboli	L. marginal		L. ventricle			+	
	31	13414	M	70	Timberman		Both ventricles	Recent infarct	Embolism, R. coronary A.	R. coro-nary A.				+	#	
D	32	6093	M	87	Sailor	500	Left ventricle	Old infarct		A.I.V.						
	33	10923	M	60	Shirtmaker	450	Interventricular septum	Septic infarct	R. coronary A.	L. coro-nary A.					+	
F	34	12529	M	81	Druggist	490	" "	Old infarct	A.I.V.		+			+		
	35	10725	M	45	Fireman	500	Saccular aneurysm of left ventricle	Myofibrosis							+	
G	36	112 GSH	F	50	Teacher		Dissecting aneurysm	Mycotic aneurysm sinus of Valsalva				Both ventricles				
	37	15672	M	66	Longshore-man	700	Left ventricle	Syphilitic aneurysm			+			+		
I	38	15603	M	72	Laborer	320	Coronary artery	Subepicardial hemorrhage		L. coro-nary A.			+			
	39	376 GSH	M	57	Waterworks	600	Subepicardial capillaries	Old aneurysm		A.I.V.						
	40	24577	M	72	Cigar dealer		Coronary artery	Subepicardial hemorrhage	A.I.V.	A.I.V.			+			

Autopsy: The pericardial sac is distended with a dark blood clot. The heart weighs 380 gm. On the left surface of the left ventricle there is a ragged perforation 1 cm. in length through which the ventricle has discharged its blood. The adjacent myocardium is of a dull yellowish brown color and has a fatty appearance, and the wall in this region is thinned by old cicatrization. Areas of fibrosis are present throughout the left ventricle. The coronary orifices are patent, the right being very small. The coronary arteries are in general extremely narrowed by sclerosis and calcification. The anterior interventricular and left marginal branches of the left coronary artery, also the right circumflex and posterior interventricular branches of the right coronary artery appear to be occluded by sclerosis or organized thrombi. The aorta shows considerable arteriosclerosis with calcification.

Histology: The microscopic picture is that of a recent infarct. The anterior descending branch of the left coronary artery is closed by what appears to be sclerotic thickening of its wall. The posterior interventricular branch of the right coronary artery is closed except for several minute recanalizations. The microscopic picture of this artery is suggestive of syphilitic arteritis. The marginal branch of the left coronary artery contains a fresh thrombus. In spite of the strong suspicion of syphilis in these arteries, infarction has evidently resulted from fresh thrombosis of the left circumflex artery.

Pathological Diagnoses: Very recent infarct with rupture in the presence of fresh thrombus of marginal branch of left coronary artery; organization and recanalization of several other coronary arterial branches due to syphilis or to old healed thrombi.

CASE 28: (Path. No. 11,716). M. W., female, obese, aged 65, a Christian scientist, who had been subject to fainting spells, died without medical attention and came to autopsy at direction of the coroner.

Autopsy: The pericardial sac is filled with clotted blood that encloses the heart. The apex of the left ventricle is adherent to the parietal pericardium by fibrous bands and in this region is found an irregular ragged tear in the thinned bulging ventricular wall producing a free communication between the ventricular cavity and the pericardial sac. On incising this portion of the wall the myocardium is found to be replaced by a thin fibrous wall. The ascending aorta contains atherosclerotic plaques and the mouths of the coronary arteries are somewhat narrowed by sclerosis. The coronary arteries are sclerotic and the anterior descending branch of the left coronary artery is occluded by a grayish thrombus. There is a dissecting aneurysm of the descending aorta. The gall-bladder has a thickened wall and contains multiple cholesterol calculi.

Histology: Microscopic examination confirms the gross finding of recent infarction implanted upon an old aneurysm of the left ventricular apex. Rupture has occurred at this site. Quite compatible with this combination of old and recent infarction is the microscopic demonstration of an old recanalized thrombus of the anterior descending branch of the left coronary artery and a fresh thrombus farther along in the course of the vessel.

Pathological Diagnoses: Aneurysm of apex of left ventricle with superimposed fresh infarct and rupture at the same site; both recent and recanalized thrombi of the anterior descending branch of the left coronary artery; chronic cholecystitis with cholelithiasis.

CASE 29: (Path. No. 13,543). E. B., male, fairly obese, aged 56, came to autopsy in the coroner's morgue following sudden death.

Anatomical Diagnoses: Recent anemic infarct and preëxisting old aneurysm of anterior wall of left ventricle with rupture; hemopericardium; arteriosclerosis of coronary arteries with calcification; fresh thrombus of anterior descending branch of left coronary artery, verified histologically; thrombosis of left ventricle; fibrinous pericarditis; anemic infarct of spleen; beginning gangrene of right foot; old amputation of left leg; chronic cholecystitis with cholelithiasis.

Comment: In this group as well as in Group A fresh coronary thrombosis was constantly found in the branch supplying the site of rupture. In addition, however, old organized and recanalized thrombi of coronary arteries were found, accounting for the old cardiac aneurysm present. The patients in this group usually have a previous history of coronary disease.

Group C. Recent Infarcts Due to Coronary Embolism (2 Examples)

CASE 30: (Path. No. 10,160). J. B., male, aged 76, a night watchman, died suddenly after breakfast and following physical exertion. He had previously been working and had not complained of illness.

Autopsy: On opening the pericardial sac the heart is found enclosed in a large blood clot. The posterior-lateral wall of the left ventricle near the apex is perforated by a ragged tear 2 cm. in length. The surrounding myocardium is hemorrhagic. The coronary orifices are freely patent. The coronary arteries are moderately sclerotic. The circumflex branch of the left coronary artery is apparently occluded by a reddish thrombus.

Histology: Microscopic examination discloses what appears to be a septic infarct. The left marginal artery contains an old recanalized thrombus. Branches of both the right and left coronary arteries contain thrombi that are probably embolic in origin. The source of these was not determined at autopsy.

Pathological Diagnoses: Septic infarct of the left ventricle with rupture in the presence of multiple thrombotic coronary emboli; recanalized left marginal artery.

CASE 31: (Path. No. 13,414). J. M. L., male, age about 70, came to autopsy in the coroner's morgue. No history was obtained.

Autopsy: The pericardial sac contains a large clot that encloses the heart. Two perforations are found in the wall of the heart. One on the posterior-lateral surface of the left ventricle is 12 mm. in length and is surrounded by several irregular reddish areas in the myocardium. The second, situated in the anterior wall of the right ventricle, measures 1 cm. in length. Atherosclerosis of the aorta is present in moderate degree. The coronary arteries are narrowed by sclerosis and calcification, and the right artery is apparently occluded by a thrombus. Sectioning of the myocardium discloses what appears to be an infarct of the left ventricle. The gall-bladder contains multiple calculi.

Histology: Microscopic examination discloses what appears to be infarction of the myocardium. The right coronary artery contains an old recanalized thrombus. In another portion of the same artery are embolic masses which appear to be loosened plaques from the arterial wall.

Pathological Diagnoses: Simultaneous rupture of right and left ventricles by separate openings; old recanalized thrombosis of the right coronary artery; embolism by loosened atheromatous plaques in another portion of the same artery; cholelithiasis.

Comment: The 2 examples in this group constitute sudden unforewarned deaths due to heart rupture following coronary embolism. In Case 30 the emboli are thrombotic and the infarct appears septic; in Case 31 the embolus consists apparently of material that had become dislodged from the lining of an artery and carried in the blood stream, in the manner described by Panum and others. This is presented in detail in a general review by one of us.⁹¹

Group D. Old Infarct and Aneurysm (1 Example)

CASE 32: (Path. No. 6093). J. A., male, aged 87, a seaman, had been hospitalized over a period of 5 months preceding death. His complaint had been shortness of breath, choking sensations and difficulty in swallowing, such that at times he had had to hold a spoon in his throat in order to facilitate deglutition. The temperature during the course of his illness had generally been subnormal, the pulse usually 80 to 90 and missing an occasional beat. The leukocyte count taken 2 weeks preceding death was 10,700. The manner of exitus was not observed.

Autopsy: The pericardial sac is distended with fluid and clotted blood. The heart, apparently of about 500 gm., is asymmetrical, the left ventricle being most prominent and roughly egg-shaped. There is a hernial bulging at the apex 4 cm. in diameter, slightly spherical, deep red in color and containing near its right margin a ragged opening that admits a 2 mm. probe. On the posterior aspect of the left ventricle 2 cm. from the first prominence is another rounded elevation which, however, is firm and unruptured. The second prominence proves on opening the heart to be an old infarct with areas of fresh hemorrhage, and from it a hemorrhage has dissected toward the thin apex, where external rupture has occurred into the pericardial sac. Diffuse fibrous pericarditis is present over the whole apical portion of the left ventricle. The coronary orifices are patent. There is considerable arteriosclerosis of the aorta and the coronary arteries are generally narrowed. The anterior descending branch of the left coronary artery appears to be occluded by an organized thrombus.

Histology: The microscopic picture is that of 2 old infarcts, 1 of them with aneurysmal thinning of the ventricular wall. A dissecting hemorrhage from the ventricular lumen has penetrated the old infarct in the left posterior wall of the left ventricle and has dissected to the apex causing rupture at that point. The anterior descending branch of the left coronary artery contains an organized thrombus with multiple recanalizations. No fresh thrombus is found.

Pathological Diagnoses: Recanalized thrombus of anterior descending branch of left coronary artery; multiple (2) old infarcts of left ventricle, 1 of them with aneurysm formation; dissecting hemorrhage through these infarcts with rupture.

Comment: In this type, as would be expected, the symptoms have extended over a period of months and have been characterized by

anginal attacks. Death has finally occurred from dissecting rupture of the chronic myocardial lesions. No fresh coronary thrombosis is present and an old recanalized thrombus accounts for the chronic myocardial lesions found.

Group E. Perforation of Interventricular Septum (2 Examples)

CASE 33: (Path. No. 10,923). O. C. B., male, aged 60, was found dead in bed in the morning. He had been ailing for some time but had not been confined to bed. He had sat up playing cards the night preceding death.

Autopsy: On opening the pericardial sac it is found to contain a little straw-colored fluid. The heart, which weighs 450 gm., is discolored posteriorly by a diffuse hemorrhage involving the interventricular septum and the posterior surface of the right ventricular wall. The pericardium is adherent at this point by fibrous bands. When the left chambers are opened a ragged rupture 2 cm. in length is seen in the muscular interventricular septum and connecting the right and left ventricles (Fig. 1). The perforation is directly below the discoloration in the posterior wall of the heart and has apparently occurred through a recent infarct.

The aortic lining is studded with yellowish plaques of sclerosis. The coronary orifices are freely patent, there being two right coronary ostia. The main branches of the left coronary artery are greatly narrowed by sclerosis and calcification. The left circumflex is occluded by an old whitish recanalized thrombus. The right coronary artery soon becomes occluded by a recent reddish thrombus extending over a distance of 2.5 cm.

On careful dissection of this heart a large, recent, mixed anemic and hemorrhagic infarct is observed in the posterior wall of the left ventricle extending into the septum, then for a short distance in the posterior wall of the right ventricle. The perforation of the septum is 4 cm. in length at its left opening and 3 cm. at its right, and extends through the septal portion of this infarct.

Histology: Microscopic examination discloses a recent infarct which, however, appears septic in type. The right coronary artery contains a fresh thrombus, evidently accounting for the large infarct in the posterior wall of the heart. The left coronary artery contains multiple recanalizations. Both of the arteries and the pericardium present a histological picture strongly suggesting syphilis, yet perforation of the septum in this case must be ascribed to infarction resulting from thrombosis of the right coronary artery in the presence of constriction of the left coronary artery due to an old recanalized thrombus.

Pathological Diagnoses: Septic infarct of interventricular septum and the posterior wall of both ventricles with perforation of the muscular septum; fresh thrombosis of the right coronary artery; recanalized thrombus of the left coronary artery; probably syphilitic arteritis and pericarditis.

CASE 34: (Path. No. 12,529). A. J., male, aged 81, died the second day after admission into Multnomah County Hospital in a stuporous condition. His sister states that he had suffered a cerebral hemorrhage one year previously, with resulting auditory and speech aphasia but no paralysis. He had complained of pain in the upper abdomen and right chest for the past 3 years, but had otherwise enjoyed fair health.

Eight days before admission the family noticed that he was pale and short of breath. On attempting to climb the stairs he was forced to sit down on the first step, and was put to bed.

Examination showed left border of heart 13 cm. from the midsternal line. The apex beat was diffuse, and the point of maximum intensity in the sixth interspace. A loud systolic murmur was heard best over the mitral area and transmitted to axilla and back. The liver was tender to palpation and 3-4 fingers' breadth below the costal margin. The temperature was subnormal, pulse 100 and weak, respiration 30 per minute, blood pressure 140/70. Red blood corpuscles 3,000,000, hemoglobin (Sahli) 80 per cent, leukocytes 11,900. The blood Wassermann was negative. Four hours before death the patient's condition became critical — the skin cold and moist, the fingernails cyanosed, the pulse imperceptible.

Clinical Diagnoses: Motor aphasia; chronic myocarditis with mitral regurgitation and acute dilatation of heart.

Autopsy: The pericardium, both parietal and visceral, is rough, red, granular and covered with fibrinous exudate, but the pericardial sac contains no free blood. All chambers of the heart are dilated. The apex of the left ventricle is thin-walled. In the distal half of the muscular septum is a ragged tear about 1 cm. in length which perforates the septum and provides a communication between the ventricles. On sectioning the septum extensive myofibrosis is found but acute infarction is not grossly evident. The lining of the perforation appears somewhat old, as if it might have existed for some time. The anterior descending branch of the left coronary artery is occluded by a recent reddish thrombus. An old area of softening 3 or 4 cm. in diameter is found in the cortex of the left cerebral hemisphere.

Histology: Microscopic examination confirms the gross diagnosis of perforation which has existed long enough to allow the formation of granulation tissue. The anterior descending branch of the left coronary artery contains a fairly recent thrombus with canalization.

Cultural Results: Culturing of the affected area of the heart in blood agar gave a pure culture of non-hemolytic streptococcus.

Pathological Diagnoses: Fairly recent perforation of muscular interventricular septum through an infarcted area in the presence of thrombus of anterior descending branch of the left coronary artery with beginning organization.

Comment: Cases of rupture of the septum occur rarely, there being about 26 in the literature, and most of these are said to have been in the membranous septum. It is of interest that both of ours are in the muscular septum.

Both are due to coronary thrombosis with infarction. In Case 33 the arteries appear syphilitic, yet syphilis cannot be considered the direct cause of rupture, as coronary thrombosis and infarction were undoubtedly present. Of interest in this connection is Pember and Stangl's⁹² report of interventricular septum aneurysm without rupture in a syphilitic subject. They attribute their aneurysm also to an old recanalized coronary thrombus rather than to syphilis.

Special interest attaches to Case 34, which was hospitalized. In this instance culture of the cardiac infarct and of the coronary thrombus yielded a pure culture of *Streptococcus viridans* and the possibility of sepsis suggests itself.

Group F. Saccular Aneurysm of Left Ventricle with Rupture (1 Example)

CASE 35: (Path. No. 10,725). W. E. J., male, aged 45, a lieutenant in the fire department, died suddenly while driving his car. Three months prior to death he had suffered from pain in the stomach radiating to the left arm, for which he consulted a physician. At that time he looked ill and suffered intensely. The heart was irregular and the pulse feeble. The blood pressure was 160 systolic. On the day of death he was on duty and feeling unusually well.

Autopsy: The pericardial sac is filled with clotted blood. Over the anterior surface of the left ventricle the pericardium and epicardium are adherent by firm fibrous tissue. On the upper antero-lateral surface of the left ventricle is a pouch-like sac about 4 by 4 by 3 cm. in diameter, whose walls are formed by the parietal and visceral pericardia and scar tissue. This pouch communicates with the cavity of the left ventricle by a narrow smooth-walled sinus only 5 by 3 mm. in diameter. The lining of this aneurysmal sac is whitish, smooth, except for radiating linear wrinkles extending outward in all directions from the opening, and continuous with attenuated trabeculae carneae and the endocardium of the left ventricle. For approximately 2.5 cm. about the opening through the ventricular wall the myocardium is the seat of patchy whitish scarring which is not present elsewhere in the heart. Like the ventricle the aneurysm contains fragments of blood clot. In the base of its inferior wall is a tear 4 mm. in length which dissects through the fibrous adhesions at the base of the pouch, finally rupturing into the pericardial sac midway between the pouch and the ventricular apex. All coronary arteries are narrowed by sclerosis and the smaller of two anterior descending branches of the left coronary artery passing toward the area of myofibrosis appears to be occluded by a white thrombus. No fresh thrombi are found. The ostium of the right coronary artery is apparently closed by sclerosis, while that of the left is greatly narrowed. The aorta, while slightly sclerotic, does not present the gross appearance usually associated with syphilis.

Histology: Microscopic examination discloses latent and healed lesions of the medial and adventitial coats of the aorta which are more compatible with rheumatic disease than syphilis. Vascularized atheromatous plaques are present in the coronary artery supplying the zone of scarring about the aneurysmal ostium, thus accounting for the gross appearance of a recanalized thrombus. The original lumen is reduced to almost microscopic size by the atheromatous process. The bulk of fibrous tissue forming the myocardial scar is obviously old, while a certain proportion of that composing the aneurysm wall gives evidence of more recent formation.

Pathological Diagnoses: Arteriosclerotic narrowing of coronary arteries; patchy myofibrosis of left ventricle with formation of saccular aneurysm, spontaneous rupture and fatal hemopericardium; focal fibrous pericarditis; atherosclerosis and probable rheumatic lesions of aorta.

Comment: The above example of heart rupture has certain points in common with Case 32, Group D, in which a dissecting hemorrhage occurred through the wall of a healed cardiac infarct. However, this case must be segregated from all other groups for the following reasons: (1) The lack of demonstrable coronary thrombosis; (2) the patchy character of the myocardial scar at the seat of aneurysm formation, more consistent with loss of muscle substance from slow ischemia as a result of arteriosclerosis than thrombosis with infarction; and (3) the unique character of the aneurysm, communicating with the ventricular cavity by a small opening.

Lack of space forbids a more detailed consideration of this form of aneurysm and we are publishing an account of it separately.*

Group G. Cardiac Rupture Due to Dissecting Aneurysm of a Sinus of Valsalva (1 Example)

CASE 36: (Path. No. 112 GSH). Mrs. D. D. W., a teacher, aged 50, died in Good Samaritan Hospital. Since childhood the patient had had chronic heart ailment. She was seen in an attack of sciatic rheumatism 4 months previous to death, and at that time looked sallow and complained of weakness, although still teaching school. Three weeks previous to death she complained of extreme weakness and had a chill, with temperature rising to 104°F. She gradually failed in strength and was admitted to the hospital, where she followed a septic course. A blood culture 24 hours before death yielded a pure culture of *Streptococcus viridans*.

Anatomical Diagnoses: Subacute bacterial endocarditis of *Streptococcus viridans* originating in a bicuspid aortic valve which had resulted probably from recurrent endocarditis; dissecting hemorrhage of right sinus of Valsalva extending upward into aortic wall, separating media and adventitia, also extending along interatrial septum and anterior wall of right auricle and ventricle, bulging into right auricle and ventricle, also bulging anteriorly into pericardial sac, with final epicardial rupture and fatal hemato-pericardium (Fig. 2); fibrinous pericarditis; extreme fatty infiltration of epicardium.

Comment: Dissecting aneurysms with rupture form too large a subject for full consideration here and for a more complete discussion the reader is referred to a previous review by one of us.⁹¹ We have had another case, however, which is similar to the foregoing example in most respects but failed to rupture. A brief report of this might be of value here.

(Path. No. 7280). P. M., single, male, a gardener, with rheumatic history and with complaint of weakness, chronic cough, difficult breathing and shortness of

* Hunter, Warren C., and Benson, R. L. Saccular cardiac aneurysm with rupture. *Am. J. Path.* (in press).

breath, died in Multnomah County Hospital. Autopsy revealed: fibrous obliteration of pericardial sac; concentric hypertrophy and dilatation of heart (weight 760 gm.); saccular aneurysm of ascending portion of aortic arch, one pouch of which involves the left posterior sinus of Valsalva, and another pouch of which dissects into the interatrial wall and bulges into the lumen of the left auricle; chronic fibrous endocarditis of left auricle; senile arteriosclerosis and syphilitic aortitis; stellate scars and miliary gummas in arachnoid over cerebral sulci.

This example differs from the preceding in that it apparently constitutes a dissecting aneurysm arising in a syphilitic aneurysm of the aorta.

Group H. Rupture of Syphilitic Cardiac Aneurysm (1 Example)

CASE 37: (Path. No. 15,672). T. P. C., male, single, aged 66, a patient in the U. S. Veterans' Hospital, Portland, Oregon, had previously been a longshoreman and ship watchman. Illness began over 2 months before death and simulated pneumonia. Patient entered the Veterans' Hospital 11 days prior to death complaining of weakness, shortness of breath and with pain around heart extending up into left shoulder. He reported that his trouble began 2 months previously with choking which awoke him from sleep and that his lungs had been "tight" ever since.

Physical Examination: Generally negative. X-ray showed enlargement of the heart in all diameters. Wassermann test was positive. A week previous to death he was troubled with palpitation and dyspnea. Examination showed temperature of 98.3, pulse rate 78 recumbent and 88 standing, blood pressure 148/90, respiration 18, no heart murmurs or arrhythmias. Blood examination showed 7300 white cells, of which 64 per cent were polymorphonuclears. For several days previous to death he felt fairly well. At 7.30 of his terminal day he refused breakfast and complained of shortness of breath. The heart rapidly failed and in 15 minutes he expired.

Autopsy: The body is well developed and 180 cm. in length. There is no evidence of a scar on the penis but the inguinal lymph nodes are palpable. The pericardial sac is adherent to the anterior surface of the heart and also to the diaphragm. When the pericardial sac is opened it is found to be filled with clotted blood. The parietal pericardium is adherent over the whole anterior aspect of the apex and on removing it the friable and greatly thinned ventricular wall comes with it, exposing a dark mass of blood clot and necrotic tissue which distends the bulging apex of the ventricle. The aorta is atheromatous and sclerotic throughout its course and shows evidence of scarring with linear wrinkling. Both coronary ostia are patent. The walls of the coronary arteries are thickened by sclerosis but not to any extent calcified. Several of the anterior and marginal descending branches of the left coronary artery are nearly occluded by grayish thickening of the walls. Coronal sectioning of the left ventricle in the region of the aneurysm and rupture discloses extensive necrosis throughout the wall of the anterior half of the apex. The myocardium is mottled, containing yellowish areas mixed with grayish, semitranslucent streaks of fibrosis and containing extensive areas of hemorrhage (Figs. 3A and 3B).

Histology: Microscopic examination of the aorta discloses irregular fibrosis of the adventitia with slight perivascular lymphoid infiltration about some of the vasa vasorum. The adjacent pericardium is more densely infiltrated with similar cells and plasma cells. The media and intima show a slight fibrous increase with beginning hyaline degeneration. There is perivascular lymphoid infiltration about a few of the vasa vasorum in the media. The microscopic picture in the coronary arteries is especially interesting and distinctive. The adventitia is densely infiltrated with lymphoid cells and plasma cells. The media is irregularly thickened by sclerosis, accompanied by considerable hyaline degeneration and with considerable distortion of the wall. In places the lumen is almost completely occluded by layers of hyaline connective tissue. In places there is dense infiltration of the media by lymphoid cells and fibroblasts and this sometimes extends through the intima to the lining of the vessel. Atheromata and calcification are almost lacking. In the smaller arteries the walls are usually moderately to greatly thickened with fibrosis of the media. Microscopic examination of the myocardium in the region of rupture shows complete necrosis usually without fibrous tissue replacement. All traces of cellular structure are lost. The margin of this large area of necrosis is peculiarly free from extensive cellular infiltration, there being only a scanty infiltration of lymphoid cells. The myocardium in other portions of the heart wall contains irregular fibrous patches of small to large size which separate the strands of thinned and atrophied muscle fibers. Perivascular lymphoid infiltration appears in the interstitial tissue in places but is not a prominent feature.

Pathological Diagnoses: Syphilis of coronary arteries with extensive syphilitic aneurysm of left ventricular apex; rupture of this aneurysm into old pericardial adhesions with fatal hemotopericardium; syphilitic myocarditis, pericarditis and mild syphilitic aortitis.

Group I. Rupture of Coronary Artery into Pericardial Sac (3 Examples)

CASE 38: (Path. No. 15,603). A. C., male, aged 72, died suddenly and reached the coroner's morgue.

Autopsy: The pericardial sac is found to be full of dark fluid and clotted blood. At the extreme apex of the left ventricle there is a diffuse blackish discoloration under the visceral pericardium. In the center of this area the surface is torn, producing a ragged slit 3 cm. in length. Sectioning shows that this rupture extends only into the subepicardial fat and not into the cavity of the ventricle (Fig. 4). The dark discoloration surrounding this is due to extravasation of blood. The ventricular wall is 2 to 2.5 cm. in thickness, except at the apex where it is only 1 cm. thick. The postero-lateral wall contains patchy areas of fibrosis but no evidence of infarction is found anywhere. Both coronary orifices are freely patent, the right being small. The ascending aorta is atheromatous but without any appearance suggesting syphilis. Both coronary arteries and their branches are considerably narrowed by atherosclerosis but without gross evidence of calcification. A branch of the left circumflex artery is occluded by an old white thrombus.

Histology: Microscopic examination of the aorta shows considerable hyaline degeneration throughout the media without any other noteworthy change. The walls of the coronary arteries are considerably thickened by fibrous increase with hyaline change in all coats. One branch of the left circumflex is almost occluded

by hyaline fibrous tissue and in the center are several small lumina. No occluding thrombi are found. Microscopic examination of the myocardium discloses a few areas of fibrosis but no evidence of infarction or any definite evidence of syphilis.

Pathological Diagnoses: Marked atherosclerosis of coronary arteries with narrowing; occlusion of branch of left circumflex artery by old recanalized thrombus. Rupture of branch of anterior interventricular coronary artery into subepicardial tissue at left ventricular apex with fatal hemorrhage into pericardial sac; no perforation of ventricular wall discoverable; patchy myofibrosis of left ventricle; atherosclerosis of aorta.

CASE 39: (Path. No. 376 GSH). W. D. C., male, aged 57, died in Good Samaritan Hospital. The patient had enjoyed fair health up to the time of a sudden attack of pain in the chest with dyspnea 4 months prior to death. Dyspnea had been severe during the few weeks preceding death. Physical examination showed ascites, also fluid at the bases of the lungs. The skin displayed a slightly icteric tint. Blood pressure registered 112/80.

The last four weeks before death were spent in the hospital, where the temperature had been constantly subnormal except during the last few days, when it fluctuated. The pulse had been mostly 100 or above, the respiratory rate usually over 40. The Wassermann was negative; white blood cells 14,800 4 weeks before death; red blood cells 5,000,000; hemoglobin (Sahli) 85 per cent. X-ray showed the heart "enlarged 50 per cent."

Autopsy: On opening the pericardial sac fibrous adhesions are encountered between the pericardium and epicardium over most of the anterior surface of the heart, dividing the sac into two parts. In these adhesions, and extending into the pericardial space to the left is a thin layer of freshly clotted blood (Fig. 5). Anteriorly the left ventricle bulges outward. The wall here is only 5-6 mm. in thickness, of cartilaginous consistence and of a pearly white color. The endocardium is greatly thickened and whitish. An organizing thrombus tampons the apex of the chamber. Cross-sections at frequent intervals fail to show a rupture of the heart wall at any point and all evidence points to rupture of capillary vessels in the pericardial adhesions as the source of the hematopericardium. There is pronounced arteriosclerotic narrowing of all coronary arteries with old recanalized thrombi nearly occluding the anterior descending ramus of the left coronary artery and two of its larger branches proximal to the old infarction.

Histology: Microscopic examination confirms the presence of recanalized thrombi in the arteries mentioned above. There is no evidence of syphilis. Capillaries and thin-walled blood sinuses of the epicardium in the region of the adhesions referred to above are found enormously distended with blood.

CASE 40: (Path. No. 24,577). W. K., male, aged 72, collapsed and died suddenly in the office of a physician whom he had consulted previously for irregularity of the heart rate following periodical drinking bouts. The blood pressure was 130/90. Nothing in the history indicated coronary disease. Just prior to death he complained of the heat, although the day was not hot, and was perspiring freely. Collapse occurred while conversing with the physician.

Autopsy: The pericardial sac contains about 100 cc. of blood which appears to have come from a small area of subepicardial hemorrhage at the apex of the left ventricle. There is no evidence of rupture although the muscle of the anterior wall of the left ventricle is soft and reddish. The lumina of the larger coro-

nary arteries are considerably narrowed by arteriosclerosis and that of the left anterior interventricular artery is occluded 2.5 cm. from its origin by a fresh thrombus situated distal to an older and recanalized thrombus. Unfortunately the heart was lost before a more detailed study could be made.

Histology: Microsections of the anterior descending branch of the left coronary artery disclose advanced atherosclerosis of the intima with great narrowing of the lumen and occlusion by a thrombus showing early organization at the periphery. In sections from the apex of the ventricle there is considerable fresh hemorrhage in the subepicardial fat but the source is not apparent. The *arteriae telae adiposae* are for the most part devoid of blood. The myocardial cells are fragmented but there is no evidence of infarction or of syphilis.

Pathological Diagnoses: Advanced atherosclerosis of coronary arteries with obstruction and occlusion of the left anterior interventricular vessel by recent thrombus showing early organization and by a recanalized thrombus proximally; probable rupture of this artery with subepicardial hemorrhage dissecting through epicardium to pericardial space; hemopericardium; atherosclerosis of aorta.

Comment: We have already given references to the literature on coronary arteriorrhesis. These are our only cases in which the fatal hemopericardium was due to escape of blood into the pericardial sac from coronary arteries. The reader will recall, however, that 2 others, Cases 4 and 12, suffered rupture of coronary arteries (multiple in Case 12) in addition to cardiac infarction. In all of our 4 cases of coronary artery rupture thrombosis of an artery seems to have been the causative factor, with the exception of Case 39, in which tearing of pericardial adhesions caused capillary hemorrhage.

DISCUSSION

The more important related facts in this extensive series of cases can best be collected in the form of a table (Table I). The clinical history, whenever available, has been given briefly in connection with each individual case report, and no further comment is necessary here.

Our series of cases present a diversity of location. Two of them represent perforations of the interventricular septum. Another constitutes an intrapericardial rupture of a dissecting aneurysm of a sinus of Valsalva. Case 39 appears to be a fatal hemorrhage into the pericardial sac from tearing of adhesions over an old aneurysm of the left ventricle. Cases 38 and 40 consist of a diffuse subepicardial hemorrhage from rupture of a coronary arterial branch with fatal leakage into the pericardial sac, the original cause apparently lying in an old organized thrombosis of a coronary branch (Case 38) and

in a recently formed thrombus in the other (Case 40). Two other examples of coronary arteriorrhaxis, Cases 4 and 12, occurred in the presence of fresh coronary thrombosis and cardiac infarction. In the first of these, rupture of the heart wall resulted with discharge of the ventricular blood into the pericardial sac, while in Case 12, thrombosis of a coronary branch was followed by dissecting hemorrhage in the wall of the vessel, which tore its way through the myocardium and epicardium with fatal hemato-pericardium.

Of the 34 cases representing rupture through the whole thickness of the left ventricular wall, 11 occurred anteriorly, 6 posteriorly, 9 laterally at the *margo obtusus*, and 7 at the apex. Two of the posterior ruptures deserve special mention. One (Case 24) began near the junction of the left ventricle and muscular septum and tore through the epicardium near the *margo acutis* after having dissected through the right posterior ventricular wall for several centimeters. Another (Case 31) posterior left ventricular perforation was accompanied by a second rupture in the anterior wall of the right ventricle.

The importance of Case 35 has already been referred to in connection with the case history, in that it constitutes the only known example of its kind. A sacculated aneurysm formed and developed a smooth lining, but finally, possibly after a long period of time, ruptured by a dissecting hemorrhage, first into pericardial adhesions and then into the pericardial sac.

Relations of age, sex, and occupation can best be obtained from Table I. Physical exertion appears to have been commonly an immediate forerunner of ruptures, as mentioned in the individual case reports.

An attempt has been made to establish definitely in each instance the condition of the coronary arteries and the consequent rôle of arterial thrombosis or embolism in distinction to syphilis or other specific infectious causes. This has necessitated careful coronal sectioning of each artery in the previously fixed heart, and has in nearly every instance been confirmed by microscopic examination of the supposed thrombus. After exhaustive histological study of the arteries and of the myocardium we feel reasonably assured that our criterion of infarction is correct in each case.

In our 25 examples of rupture of a recent infarct which have been grouped together under Class A, we have demonstrated the throm-

bolic occlusion of the coronary artery in all except the heart in Case 11, which was lost before examination was completed. Some of these hearts have indeed manifested microscopic evidence of syphilis of the coronary arteries, myocardium, and aorta, as noted in the table, but even in these instances unquestioned thrombosis of the coronary vessels has been proved.

Likewise, in the 2 infarct examples, Cases 30 and 31, the immediate cause has been demonstrated in the form of emboli. Again in the 4 cases of Group B, those having a recent infarct on the basis of a preëxisting cardiac aneurysm, the recent arterial thrombus has been found, usually along with evidence of an earlier organized thrombus of coronary arteries. In another heart, Case 32, an old infarct had ruptured, and, as might be expected, an old organized thrombus of a coronary artery was found. In the two interventricular perforations, Cases 33 and 34, we have demonstrated coronary thrombosis as the forerunner of the cardiomalacia. The probable genesis and atypical character of the aneurysm in Case 35 render this case so unique that it is being described in a separate paper.* In the 3 remaining instances of coronary arteriorrhaxis, Cases 38, 39 and 40, we have likewise demonstrated old organized coronary thromboses. Thus, 38 of our series of 40 appear to be examples of cardiorrhaxis attributable to recent or old coronary occlusion by thrombi, emboli or atherosclerosis.

The 2 cases falling outside of this coronary occlusion group are 36 and 37, and of these Case 36 obviously represents the dissection of a mycotic aneurysm of the aortic valve due to *Streptococcus viridans*. The remaining specimen (Case 37) appears syphilitic, and the reader is referred to the detailed record for evidence to that effect. This subject gave a positive Wassermann and the heart and aorta bore typical evidences of syphilis. It is reasonable to assume that in this instance syphilis was probably responsible for the rupture.

We have already mentioned that several other hearts in the series showed microscopic evidences of syphilis in addition to the more certain presence of coronary thrombosis. We cannot admit, however, that syphilis is responsible for the cardiorrhaxis unless indeed it can be allowed that this infection is a common precursor of coronary thrombosis. Such a relationship has never been established.

* Hunter, Warren C., and Benson, R. L. Saccular cardiac aneurysm with rupture. *Am. J. Path.* (in press).

Finally, attention is called to the high incidence, 17.5 per cent, of cholelithiasis in our series.

SUMMARY

Forty ruptures of the heart have been collected from nearly 7000 autopsies in Portland, Oregon. One of these was probably of syphilitic origin. Another, a dissecting aneurysm of a sinus of Valsalva, was due to *Streptococcus viridans* endocarditis. The remaining 38 ruptures, although in some instances manifesting evidence of syphilis, were attributable to recent or old thrombosis, embolism or arteriosclerosis of the coronary arteries.

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DESCRIPTION OF PLATES

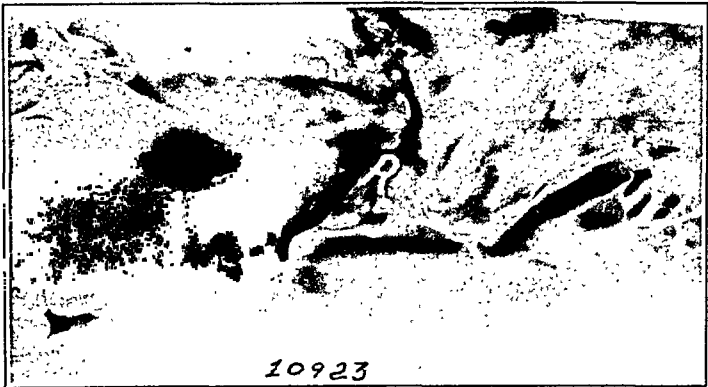
PLATE 51

FIG. 1. Case 33. Sectional view of septum. Natural size.
R = Site of rupture.

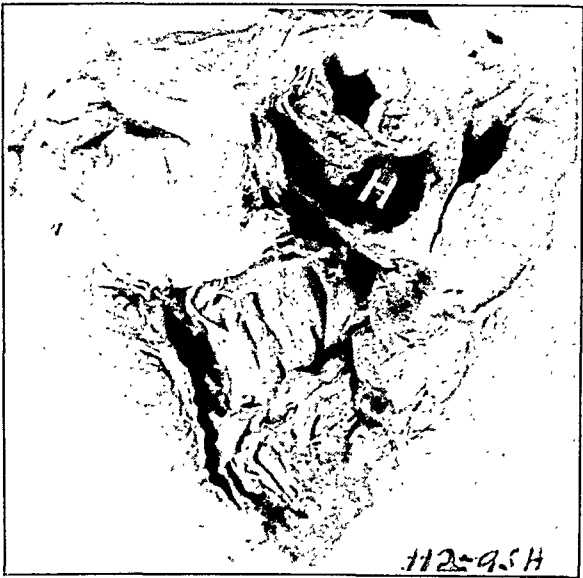
FIG. 2. Case 36. Intrapericardial rupture of dissecting hemorrhage from sinus of Valsalva due to *Streptococcus viridans* endocarditis. Right ventricle opened.
A = Aortic valve.
H = Dissecting hemorrhage.

FIG. 3 A. Case 37. Coronal section through site of rupture.
R = Site of rupture.
N = Necrotic area.
T = Intraventricular thrombus.
A = Wall of aneurysm.

FIG. 3 B. Case 37. Coronal section proximal to rupture.
H = Dissecting hemorrhage.
N = Necrotic area.
T = Intraventricular thrombus.



I



2



PLATE 52

FIG. 4. Case 38. Rupture of coronary branches with resulting subepicardial hemorrhage (H) and final rupture into pericardial sac.

FIG. 5. Case 39. Hematopericardium due to tearing of pericardial adhesions overlying old healed aneurysm of left ventricle.

P = Parietal pericardium, reflected back, showing torn adhesions.

T = Portion of intrapericardial thrombus.



4



5

A COMPARISON OF THE CELLULAR REACTION IN EXPERIMENTAL TUBERCULOSIS OF THE CORNEA IN ANIMALS OF VARYING RESISTANCE *

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Previous experiments by Vorwald¹ have shown that a definite correlation does not exist between the intensity of cellular reaction in the lung in different animals after implantation of tubercle bacilli, and the subsequent development of tuberculosis in this organ. His studies at the same time indicated that a large percentage, perhaps the majority, of the cells taking part in the formation of the developing tubercles under the conditions of his experiment came from the blood stream. A study by Long, Vorwald and Donaldson² on the early cellular reaction to tubercle bacilli in the testis in normal and tuberculous guinea pigs and guinea pigs immunized with dead bacilli, led to the same conclusions.

Vorwald's observations brought out again with unusual clarity the facts in the course of tubercle development first described by Borrel³ and his associates, and recently emphasized by Albert-Weil,⁴ *viz.*, the immediate influx of polymorphonuclear leukocytes at the point of lodgement of tubercle bacilli and the subsequent phagocytosis and replacement of these cells by large mononuclear cells. The apparent vascular origin of most of the cells concerned suggested the value of a similar study of tubercle formation in different animals in the non-vascular cornea. It seemed possible that differences in the rate of tubercle growth in different animals might be still more conspicuous in the latter organ.

Four series of animals were inoculated with human type tubercle bacilli (H 37 of Saranac Lake), *viz.*, normal and tuberculous guinea pigs, rabbits and cats. Interlamellar injections were made with sharp, short beveled, fine gauge hypodermic needles in or near the center of the cornea in each case. It was impossible to fix the dosage exactly because of the minuteness of the amount of fluid that could be injected into the cornea without detrimental trauma or uncon-

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trolled spreading, but the approximate amounts of tubercle bacilli injected were 0.01 mg. in the rabbits and cats, and 0.005 mg. in the guinea pigs. A sudden, sharply limited, white opacity 2-3 mm. wide in the cornea in the region inoculated, on slight pressure of the loaded syringe after the needle was in place, signalized the success of the injection.

The course of development of the infection in the different animals is summarized in Figure 1. The relative sizes of the lesions are shown by fine shading at the site of infection, and blood vessels are indicated by small circles, at the limbus in all animals and at the site of infection in the sensitized guinea pig killed at 2 weeks and the normal guinea pig and rabbit killed at 1 month. Approximate simple differential counts are given of the cells partaking in the reaction, the region at which the count was made being indicated by an arrow in each case.

At 6 hours no reaction was apparent in any animal except at the limbus. After the passage of 24 hours a cellular reaction was seen in the injected area, in or bordering on the center of the cornea, in every animal. In the figure attention is drawn to the limbus reaction at the early and to the central reaction at the later periods.

Significant facts from observation of sections, including those stained for tubercle bacilli, are as follows, and the following conclusions seem warranted in their explanation.

1. The first cellular response apparent in the infected cornea was around the blood vessels of the limbus in every instance; it was notable here 6 to 24 hours before a reaction could be detected at the actual site of injection. This seemed good evidence that the cells responding immediately to the infection came from the blood vessels at the margin of the cornea, and not from the conjunctival secretion along the needle track, as was claimed by many of the early observers.

2. A considerable variation in the limbus response was seen in the different animals of the series at the 6 hour period. The most intense reaction occurred in the sensitized (tuberculous) guinea pig. The severity of reaction in the normal guinea pig, rabbit and cat decreased in the order named. The prompt outpouring of cells in the already infected guinea pig appeared simply another example of the well known hypersensitivity in this type of animal. It could quite appropriately be considered a tuberculin reaction, and indeed other

experiments by ourselves have shown that the same divergence in intensity of reaction in the cornea occurs in normal and tuberculous guinea pigs following the intracorneal injection of purified tuberculin protein. The difference in intensity of initial reaction in the normal animals was similar to that observed by Vorwald in the lung, and is further evidence that intensity of reaction at the outset does not predicate corresponding speed of suppression of the infection. On the contrary, the least reaction was observed in the cat, the animal of the highest general resistance to infection.

3. The predominant cell in the limbus reaction at 6 hours, the earliest period studied, was the polymorphonuclear leukocyte in all instances. At 24 hours cells of this type had reached the point of inoculation in large numbers, whereas few mononuclears had yet appeared at this point. Large mononuclears were present at 24 hours in considerable number, however, in the zone of reaction at the limbus. As time went on, as shown by sections at later periods, mononuclears gradually increased in number in the central zone of reaction at the site of infection, and rapidly increased when blood vessels reached this point. These facts, taken together, seemed to warrant the conclusions that (*a*) both the polymorphonuclear leukocyte and the large mononuclear in the developing tuberculous lesion in the cornea come from the vascular system, and (*b*) the former reaches the infected region sooner because it is a more rapidly moving cell. The failure of large mononuclears in the early reaction at the site of infection seems good evidence that the local cells do not partake in the reaction, as claimed by Baumgarten⁵ in his early work.

In passing it should be said that objection may be made to these conclusions because of the size of the dose of tubercle bacilli used in the experiments here recorded. Krause⁶ has stressed the point of dosage in the following words: "If we eliminate all disturbing factors that may arise through the inoculation of too large quantities of bacilli we may arrive at a true appreciation of the character of the process which gives rise to the aggregation of epithelioid cells which form primary tubercle. In response to the localization of a very few bacilli and during the first few days after localization, we find that not a vestige of tissue disturbance occurs except at and around the immediate point of localization of bacilli. Between this site and any other point we cannot trace the slightest evidence (anatomical) that the bacilli have exerted the least effect on any other cells than those

of the immediate neighborhood. Save for the completely isolated aggregation of epithelioid cells, enclosing tubercle bacilli, the microscopic field discloses entirely normal tissue — of lung or cornea for instance. At the same time, within the epithelioid masses cells in active mitosis are frequently to be observed. These two phenomena compel the opinion that the *origin* of nodular tubercle, that is, the first aggregation of epithelioid cells, is accomplished through the *proliferation*, in other words the new growth, of cells at or near (within microscopic distance of) the point of localization of tubercle bacilli, and normally existing there.”

The question of the origin of the cells entering into the reaction will be considered in detail, in connection with further experiments, in the next chapter.

In explanation of the size of the dose used in our experiments we should say that we desired to follow the fate of the tubercle bacilli injected, and for this reason injected a quantity making this effort easy. The amount injected is not excessive according to the usual standards for the inoculation of tubercle bacilli. Nevertheless, in order to answer the question of relation of type of reaction to dosage we diluted the suspension of bacilli used in the experiments here reported 100 times for an additional, otherwise similar experiment in the rabbit cornea. It is significant that although the intensity of reaction was very much reduced, qualitatively no difference was observed from that seen and here described with the larger dose. Accordingly we felt that valid conclusions for the general subject of tuberculous infection could be drawn from our more extensive experiments with the doses of 0.005 to 0.01 mg. of bacilli.

4. The tubercle bacilli injected were practically all phagocytosed by polymorphonuclear leukocytes within the first 24 hours. With the arrival of the large mononuclear phagocytic cells, ingestion of the polymorphonuclears by these cells occurred, with a corresponding transfer of the tubercle bacilli first taken up by polymorphonuclears. The situation was like that described and illustrated by Vorwald, except that in the present case the arrival of the large mononuclears was a much delayed process. At 72 hours the predominant cell at the site of infection in all animals was still the polymorphonuclear leukocyte, but at the two later periods studied, 2 weeks and 1 month, the large mononuclears were in considerable excess, and the bacilli were chiefly within their cytoplasm.

5. The final picture showed characteristic differences. The sensitized guinea pig was dropped from the series recorded in the accompanying figure, because at this period in reinfected guinea pigs ulceration occurred and heavy secondary infection complicated the picture. In the other animals the intensity of the reaction, as measured by the proportion of the cornea in a state of inflammatory reaction, varied in the guinea pig, rabbit and cat, with decrease in the order named. At 1 month vascularization of the infected area was well developed in the guinea pig and rabbit, and in these animals the cells of the lesion were now predominantly mononuclear. In two out of three cats, on the other hand, vascularization of the infected area had not yet occurred and the proportion of mononuclear cells of inflammation was much lower. As the cat had the highest general resistance to infection with the strain of tubercle bacillus used, and the guinea pig the least, the intensity of reaction at 1 month, the period of full tubercle development, was seen to be in inverse proportion to the general resistance.

SUMMARY

Central interlamellar corneal injections of 0.01 to 0.005 mg. of moderately virulent tubercle bacilli of human type were made in a series of normal and tuberculous guinea pigs, and normal rabbits and cats. Animals of each kind were killed at 6 hours, 1, 3 and 14 days and 1 month, and the nature and extent of the inflammatory response determined by microscopic examination.

In all cases the first reaction noted was at the limbus, and not at the site of injection. The reaction consisted of an outpouring of polymorphonuclear leukocytes from the marginal vessels. It was most intense in the tuberculous guinea pig (tuberculin reaction), less in the normal guinea pig, still less in the rabbit and least in the cat.

The first cellular reaction at the site of injection in the center of the cornea consisted of leukocytes migrating in from the margin. The reaction was well developed in all animals at 24 hours, and the cells taking part were almost exclusively polymorphonuclear leukocytes.

After 24 hours there was a gradual increase in the proportion of large mononuclears in the reaction. The rate of increase rose with the closer approach of blood vessels, as vascularization of the cornea

developed. The paucity of these cells at the earlier periods and abundance in the vascularized state of the cornea seemed good evidence that the large mononuclear leukocyte in corneal tuberculosis is not locally derived, as formerly claimed. In all animals the large mononuclears, as they reached the site of infection, engulfed the polymorphonuclear leukocytes already there, taking over the tubercle bacilli contained by the latter cells.

From 3 days to 2 weeks the inflammatory reaction progressed most rapidly in the tuberculous guinea pigs, and less rapidly in the normal guinea pigs, rabbits and cats in the order named. Ulceration occurred in the tuberculous pigs in 2 weeks, and these pigs were dropped from the series at this point.

At 1 month, the last period studied, the intensity of inflammatory reaction, as determined by the size of the lesion and proportion of the cornea involved, was greatest in the guinea pig, less in the rabbit and least in the cat. This order is in inverse relation to the general resistance of these animals to the strain of tubercle bacillus used.

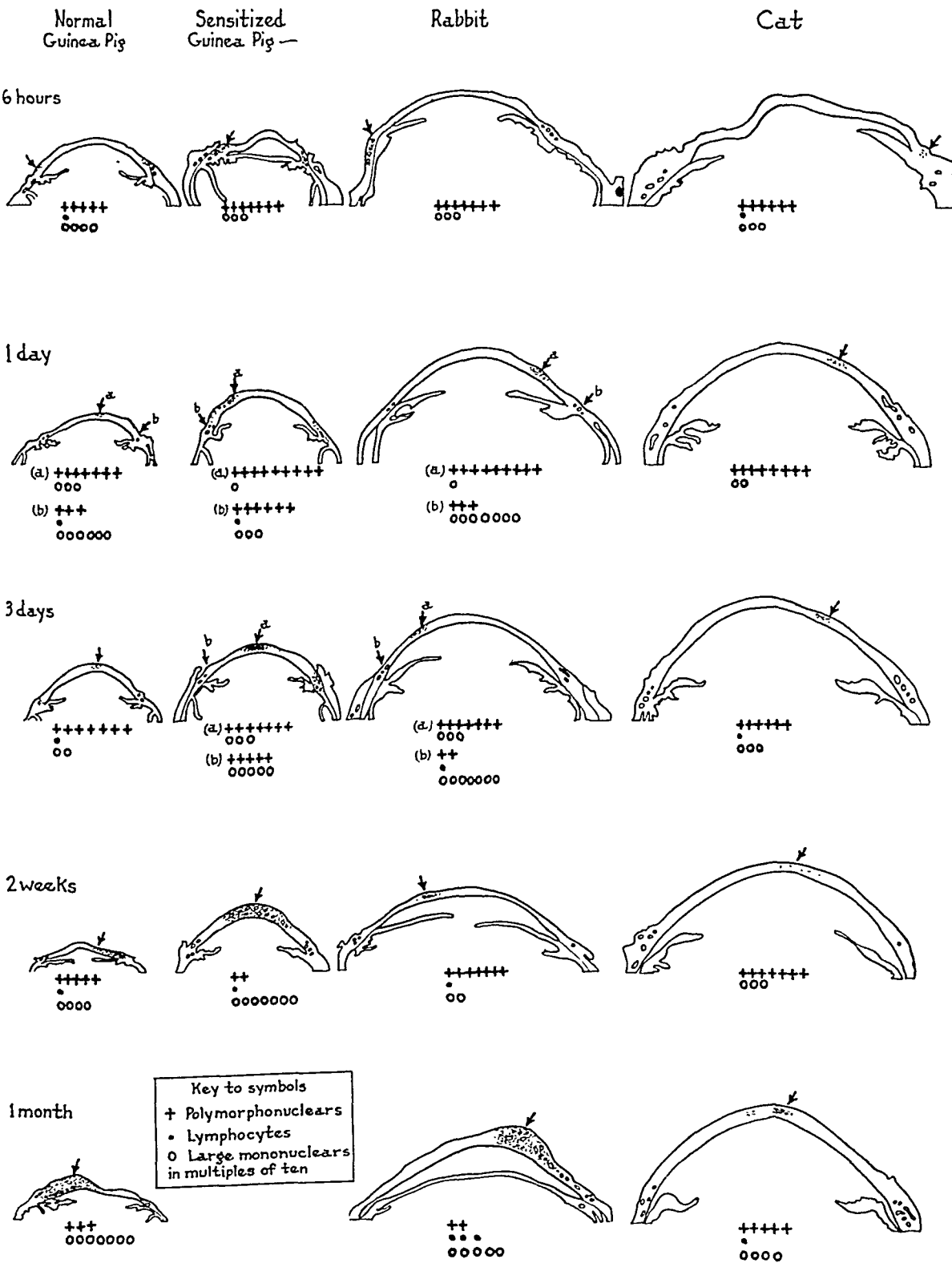
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DESCRIPTION OF PLATE

PLATE 53

FIG. 1. Course of development of tuberculosis of the cornea in different animals. The size of the lesion is indicated by fine shading. Blood vessels are indicated by small circles and ovals. Approximate differential counts of the cells are given, the regions at which the counts were made being marked by arrows.



THE ORIGIN OF THE EPITHELIOID CELL IN EXPERIMENTAL TUBERCULOSIS OF THE CORNEA *

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The cornea has frequently been used in the experimental study of inflammation. Surrounded by capillaries, but normally avascular itself, it is an ideal site in which to study the movements and focalizations of wandering leukocytes. It is therefore not surprising that even fifty years ago it was employed in the attempt to determine the origin of the characterizing element of the tubercle, the so-called epithelioid cell. In 1885 Baumgarten¹ undertook a study that has formed the starting point for numerous subsequent investigations. Even at this early date there was dispute as to whether the characteristic cells of the tubercle were derived from the blood stream or from fixed tissue elements.

Baumgarten, using a procedure already fruitful in the hands of Cohnheim and Koch, planted bits of tuberculous tissue in the anterior chamber of rabbits' eyes and watched the course of development of tuberculosis in the adjoining structures. In a second series of animals he inoculated the cornea directly with suspensions of ground tuberculous organs. He distinguished two types of reaction, (1) an immediate acute one, marked by rapid influx of wandering cells; and (2) one occurring several days later around new foci of multiplying bacilli, characterized by mitoses among the fixed tissue cells, and subsequently by the appearance of cells of an epithelioid type. The first reaction he thought was due to trauma as well as to the bacilli injected, and he was of the opinion that the greater part of the cells entered from the conjunctival fluid. The second he looked upon as the characterizing response of tuberculosis. His conclusion was that the typical epithelioid cell of the tubercle in the cornea is a derivative of descendants of the fixed tissue cell, and not a wandering leukocyte. He held the same view in regard to the epithelioid cells in tuberculosis of other organs.

* Received for publication January 12, 1933.

Although not unchallenged, this conception remained dominant until recently. For a clear epitome of the Baumgarten view the reader is referred to Krause,² who adopted it, with certain reservations, in his exposition of the anatomical structure of tubercle from histogenesis to cavity. More recent studies in hematology and cytology, however, have thrown new light on the problem, and at the same time have shown it to be much less simple than was formerly supposed. Among these are the extended studies of Sabin and her coworkers on experimental and clinical tuberculosis, and experimental tubercles produced by the injection of purified substances from the tubercle bacillus. Using the supravital technique Sabin, Doan and Cunningham³ traced the origin of the epithelioid cell to the monocyte, pointing out that the latter is a cell both of the connective tissues and of the circulating blood. The extensive studies of Maximow⁴ and Bloom⁵ on the transformations of lymphocytes and monocytes have shown that epithelioid cells may develop by a series of changes from stem cells, both of the blood stream and of local tissue. Although common ground can be found in the positions of the different investigators in the field, a good deal of confusion has developed as the result of attempts to make sharp distinctions on the basis of source and function between the various large mononuclear, potentially phagocytic cells. Recently Sabin⁶ in reviewing the conflicting evidence on relationship between the monocytes and macrophages, as developed by herself and her colleagues, and by Maximow, Bloom, M. R. and W. H. Lewis, and Carrel and Ebeling, has stated the reconciling conception toward which there now seems a general trend. This is, briefly, that the monocyte and clasmatoocyte are merely stages of the same cell, their differences depending on the material they have phagocytosed rather than on differences in cytoplasm adapting them to different functions. Her own studies have indicated that the typical monocyte of the connective tissues and blood is a younger type of cell than the typical clasmatoocyte of the connective tissue. The recent experimental studies of Hetherington and Pierce⁷ on the transformation of cells of the monocytic class into typical tissue macrophages and further into epithelioid and giant cells, bear out this view.

The cornea seems especially well suited to further investigation of some of the puzzling questions thus raised. Previous studies⁸ have shown that injection of tubercle bacilli into the cornea of rabbits and

other animals is followed, as in other organs, by an accumulation first of polymorphonuclear neutrophile leukocytes around the bacilli, and then of large mononuclear wandering cells which engulf and replace the leukocytes. It seemed significant that arrival of the large mononuclear cells approximately coincided with the appearance of capillaries in the injured, previously avascular area. This observation made it seem probable that the large mononuclear cells, which ultimately gave rise to epithelioid cells, came either from the blood stream directly, or from the walls of the new ingrowing blood vessels, and not from local fixed tissue cells of the cornea.

In order to test this hypothesis an experiment was devised for comparing the development of tuberculous areas in the same eye, but respectively near and far from the original blood supply. It seemed probable that if the source of the epithelioid cells was the vascular system a region of infection near the limbus would become of epithelioid character more rapidly than one in the center of the cornea. Accordingly in each of a series of large rabbits two infections with 0.01 mg. human type tubercle bacilli (H 37), of low but still appreciable virulence for this animal, were introduced in the same cornea, one about 2 mm. from the limbus, the other in the center or 5 to 6 mm. from the limbus. Animals were killed at 1, 6, 14 and 48 days. The infected eyes were fixed in formol-Zenker's fluid, and the corneal half of each was embedded in celloidin. The blocks were mounted in such a way that the plane of sectioning included the site of both infections and the intervening tissue. Three sets of sections were prepared, one stained with hematoxylin and eosin, one with hematoxylin, carbol fuchsin and orange G, and one with eosin-azure. By the second technique the tubercle bacilli and the granules of the polymorphonuclear leukocytes were well shown. The third technique was used in attempting to differentiate the mononuclear leukocytes.

The experiment yielded the results anticipated. Representative fields from the two sites of infection at each period are shown in the accompanying figures.

At 1 day the cell responding to the infection was the polymorphonuclear neutrophile leukocyte. Cells of this type were much more numerous at the limbus, close to the blood supply, but the reaction at the center, although less intense, was qualitatively the same, and thin lines of leukocytes could be seen streaming from the

limbus between the connective tissue fibers, all the way to the center, where they had formed a small focus around the bacilli.

At 6 and 14 days the animals yielded mutually similar results. In each a significant difference was now apparent between the reactions near and remote from the limbus. Near the limbus the invading cells, which at 1 day were almost all polymorphonuclears, were now mostly large mononuclears. Occasionally migration figures of large mononuclear cells could be seen in the walls of the blood vessels (Fig. 3). New capillaries could be found in this region, larger and better formed close to the limbus, but not yet within 2 to 3 mm. of the other site of infection at the center. In the latter non-vascular region the reacting cells were still chiefly polymorphonuclear leukocytes, although a few lymphocytes and large mononuclears were to be seen. At intermediate points, where vascularization was more or less advanced, a mixture of the two cells was found.

At 48 days another significant change was apparent. The reaction in the outer zone, near the limbus, was still almost exclusively of mononuclear character, but the cells were of a more mature type, including large aggregations of typical epithelioid cells and many fibroblasts with associated collagenic fibers. The cornea in this region was now at least double its original thickness. Some spots of necrosis had developed. In the center the reaction, although less intense, was qualitatively similar to that near the limbus. Vascularization was well developed from the periphery to the center of the cornea. Polymorphonuclear leukocytes had practically disappeared and in their place there were large mononuclear wandering cells in varying stages of maturity.

Wherever the cellular response was characterized by the appearance of large mononuclears, *i. e.* near the limbus in the 6, 14 and 48 day rabbits, and at the center in the 48 day rabbit, these cells were present in greatest concentration around the blood vessels. From this observation alone it could not be determined whether they were derived from the lumen or the wall of the vessel, but several other features of the reaction threw further light on this question. The characteristic arrangement of cells in the newly developed tissue is shown in Figure 7. Typical and atypical vessels could be distinguished. Commonly, as shown in more detail in Figures 5 and 6, the new vessels were surrounded by a collar of large mononuclear cells. In hematoxylin-eosin and eosin-azure sections the nuclei were seen

to be rich in chromatin, and spherical or oval, with only a slightly longer axis in one direction, or, more rarely, irregular with a slight tendency to bilobate form. The cytoplasm was small in amount, but somewhat more extensive than in normal lymphocytes, and basophilic. Cells similar to these were also found mixed in with red cells within the lumina of these vessels. Although the total number of such cells within the vessels was small as compared with the number outside, it was in great excess of the normal, and migration figures derived from such cells could be seen in the vessel walls.

In Table I are given counts of cells in and around cross-sections of three new capillaries. As these counts are representative of the scores of young capillaries present, no more need be given. It is apparent that the ratio of mononuclears to erythrocytes is much higher than in normal blood.

TABLE I

Cell Counts In and Around Cross-Sections of Three New Capillaries

Cells in the vessel lumen			Cells in and around the vessel wall
Erythrocytes	Mononuclears	Polymorphonuclears	Mononuclears
37	8	1	41
19	2	1	10 (one typical migration figure)
56	6	1	79 (including several apparently passing through the endothelium)

Atypical vessels filled with large mononuclears similar to those in the perivascular collars just described, were scattered throughout the new inflammatory tissue, as shown in Figure 7. One is shown in detail in Figure 5. In some instances these were lined by a typical layer of endothelium; in others a thin eosinophilic fibrillar membrane without nuclei formed the limiting wall. In other cases cells of identical character, free of endothelial or other limiting membrane, lay in sharply circumscribed masses, circular or oval in cross-section, within tissue spaces. On examination of serial sections many of these masses of cells could be traced back to the perivascular collars. All of these structures appeared to be simply developing branches

from the new capillaries, equipped with endothelium close to the vessel, and without it at a distance from the vessel. At some distance the large mononuclears seemed to be simply pushed along into tissue spaces.

Outside of the perivascular collars of chromatin-rich, large mononuclear cells, transitional forms of these cells could be recognized. These may be seen in Figures 5, 6 and 7. The nuclei were in general character similar to those seen in the perivascular collars and in the extensions from them described above, but certain important changes were apparent. The nuclei were larger and the chromatin was more widely dispersed. In the perivascular collars the chromatin granules were large and close together. In the cells a small distance away the chromatin granules were of similar size but more widely separated. The cytoplasm was less basophilic. A considerable variation in size and degree of change from the perivascular cells could be distinguished. Still further away from the new vessels a rather abrupt change took place to a type of cell with a larger, pale nucleus of less granular character and an eosinophilic cytoplasm. In the zone of transformation mitoses could be seen, but only rarely. The extreme degree of change was to a cell of typical epithelioid character. Sharply circumscribed areas of degeneration and necrosis with pyknosis and karyorrhexis occurred within masses of such epithelioid cells. In other words characteristic tubercles had formed. Only giant cells were lacking to make the typical picture complete.

In brief summary the process of chronic inflammatory reaction in the cornea seemed to be as follows.

1. Vascularization of the infected region.
 2. Appearance of primitive mononuclear cells in (*a*) blood vessel lumina and (*b*) blood vessel walls.
 3. Appearance of primitive mononuclear cells in perivascular arrangement.
 4. Transformation through several stages to similar cells radiating from the perivascular accumulations.
 5. Further transformation to typical epithelioid cells undergoing degeneration and necrosis in circumscribed areas.
- The new tissue thus appeared to be made up of new blood vessels

and the perivascular mononuclear cells and their derivatives. The key to understanding of the histogenesis of the new inflammatory tissue seemed to lie in the conspicuous perivascular cellular collars, as these were evidently the source of all the new tissue outside of all the blood vessels.

Three observations seemed significant in determining the origin of these perivascular cells. First, similar cells could be seen in the vascular lumina, as shown in Figure 14. Second, similar cells could be seen occasionally caught by the process of fixation in the act of migration through the vessel walls. Several of these are shown in Figures 9-13. The number of such migration figures was not large, but probably represented only a small fraction of the migrations actually taking place at the time of the fixation. Experimental support for this view is found in the effect of small amounts of fixing fluid on the migration figures of tissue culture. The admixture of minute drops of Zenker's fluid with a growing culture causes prompt rounding up of moving cells with contraction of all their pseudopodia (unpublished observations of Aronson). In the sections under consideration here many mononuclear cells were found adherent to the walls of the capillaries, which might have been in the process of migration when the warm material was transferred from the animal body to the fixing fluid. The third observation of importance was the rarity of mitotic figures in the dense perivascular cellular collars. Occasional mitoses were indeed observed, but they were less than one-tenth as numerous as the migration figures.

All these facts taken together led us to believe that the chief source of the mononuclears surrounding the young capillaries was the blood stream, rather than the fixed tissue cells of the vessel wall. The mitoses rarely observed around the capillary walls were perhaps evidence that the fixed cells of this site participated to some extent in the formation of the perivascular collars. They seemed, however, a much less frequent source than the blood stream.

It has not seemed advisable in this paper to attempt to designate the important cells considered other than as primitive mononuclear cells. The interpretation of hematoxylin-eosin and eosin-azure stains varies with different observers, and supravital staining, if not subject to the same criticism, was impossible here because of the necessity of preserving topographical relations. The relationship of these cells to primitive lymphocytes and monocytes is thus left unsolved.

They appeared to be entirely different from the typical clasmatoocyte, which has a more abundant cytoplasm. Cells of the latter class also were observed in small numbers in the perivascular collars, and the mitoses found occasionally among the cells outside of the collars appeared traceable to them. The cells forming the greater part of the perivascular mass were smaller and characterized by much denser nuclear stain. Their transformation to cells of an epithelioid character appeared to come about through progressive increase in size and change in character of the original cells, without the intermediary process of mitosis. This sort of change has been described by Maximow⁹ as a "rapid hypertrophy" in his exposition of the transformation of the monocytic cells of the blood stream into large phagocytes. The cells seen in the massive chronic inflammatory reaction here described appeared to attain their ultimate epithelioid character gradually, not only without mitotic division but also without the formation of distinctive intermediary cell types.

SUMMARY

1. Infection of the center of the rabbit cornea with tubercle bacilli of human type resulted in a lesion characterized almost solely by polymorphonuclear leukocytes until the arrival of new capillaries, when a rapid replacement of the polymorphonuclears by mononuclear phagocytic cells occurred. When two infecting doses were placed in the same cornea, one at the margin near the normal blood supply and one in the normally avascular center, the replacement of polymorphonuclears by large mononuclears occurred weeks earlier in the lesion close to the limbus.

2. The mononuclears accounting for this replacement and functioning in the subsequent development of the lesion apparently arose from primitive smaller mononuclear cells present in large numbers in and around the walls of the new capillaries. The source of these cells could not be determined with absolute certainty, but the following observations were evidence that most of them came from the blood stream: (a) cells of similar character were present among the erythrocytes in blood vessel lumina in great excess of the normal proportion; (b) migration figures of similar cells could be seen in the walls of the growing capillaries, and (c) mitotic figures among them were rare.

3. The primitive cells after accumulating in and around the capillary walls underwent a progressive transformation by simultaneous increase in size and change of character, without mitosis, into epithelioid cells.

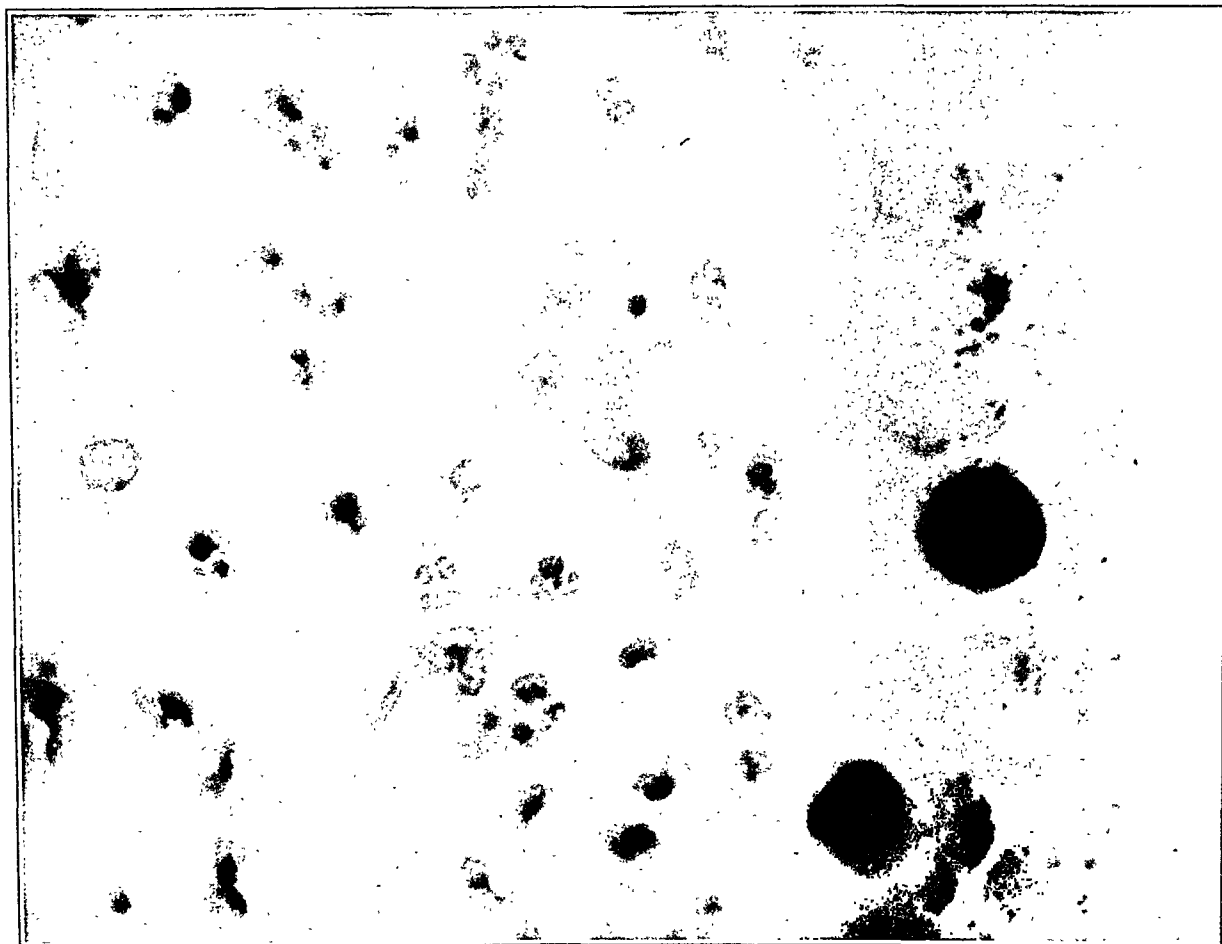
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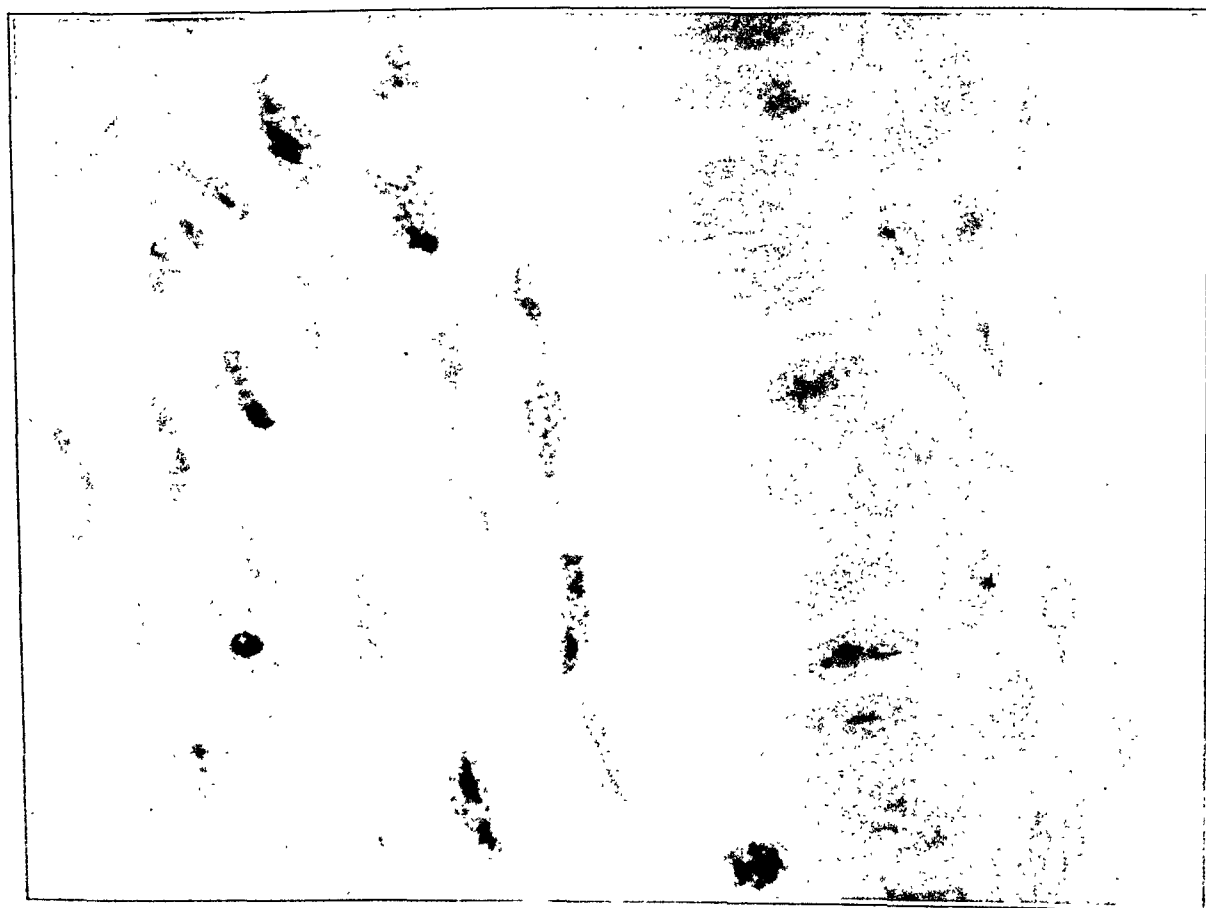
DESCRIPTION OF PLATES

PLATE 54

- FIG. 1. Cornea of rabbit 24 hours after double injection of tubercle bacilli. Section from site of injection at margin. Note preponderance of polymorphonuclear leukocytes in inflammatory reaction. $\times 1200$.
- FIG. 2. Cornea of same rabbit 24 hours after double injection of tubercle bacilli. Section from site of injection at center. Note preponderance of polymorphonuclear leukocytes, which in this section are heavily stained for the granules. $\times 1200$.



I

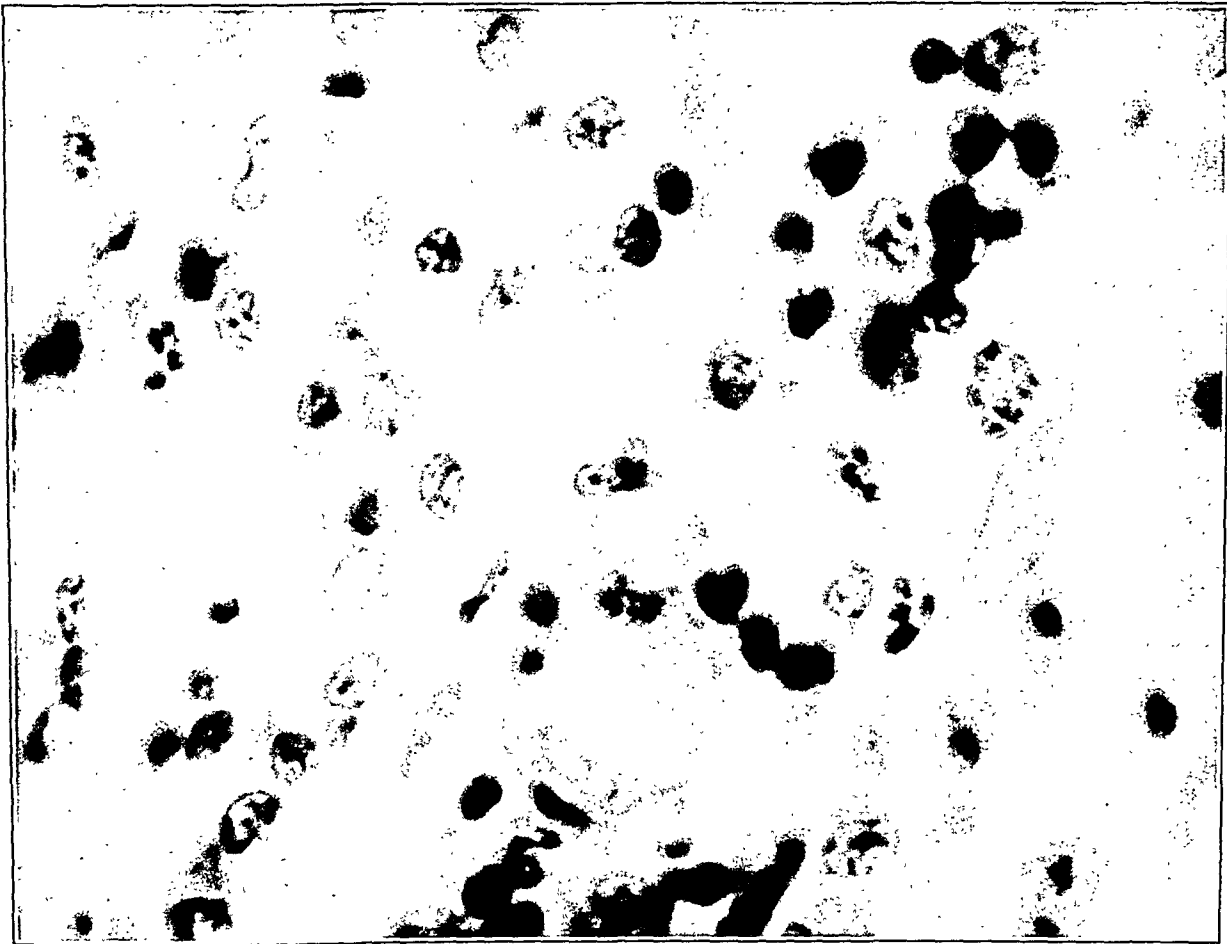


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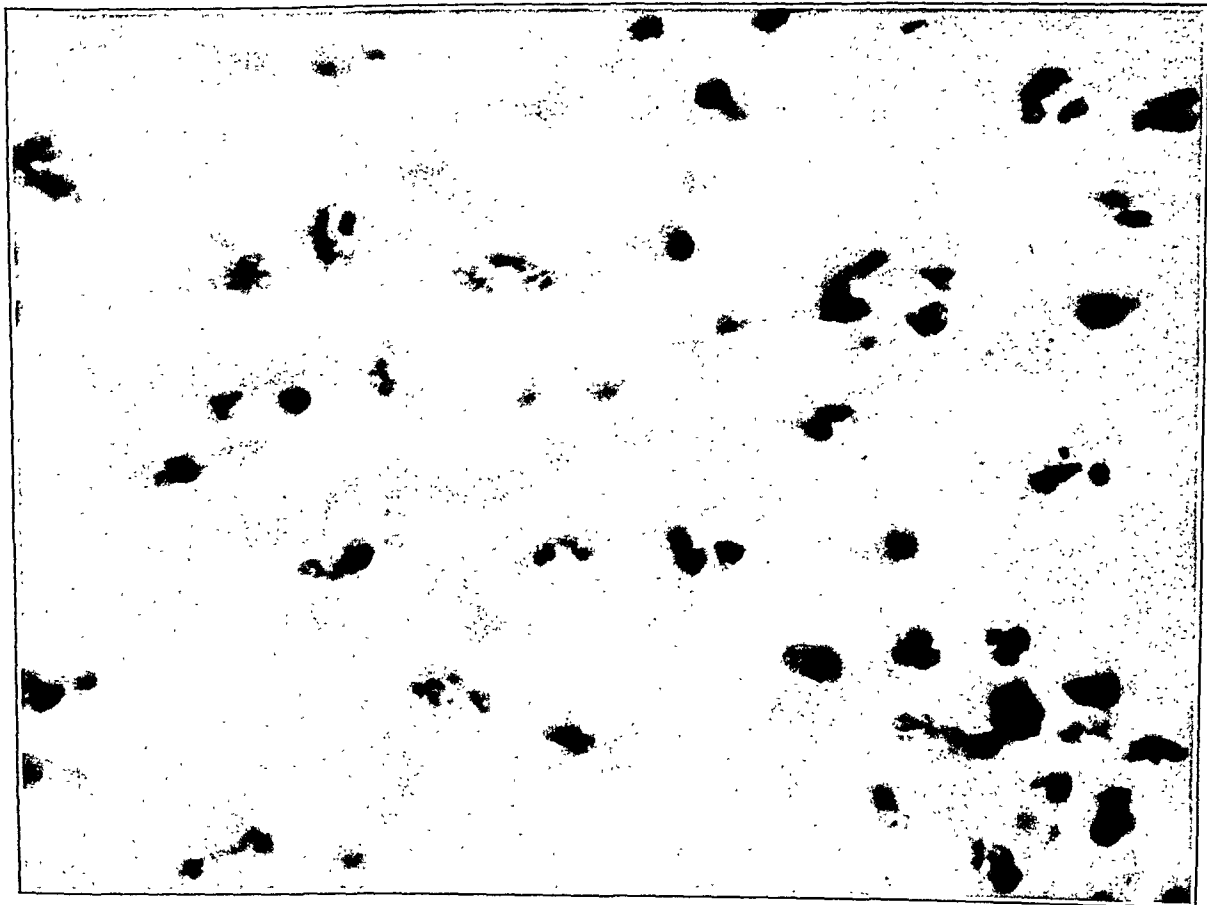
PLATE 55

FIG. 3. Cornea of rabbit 2 weeks after double injection of tubercle bacilli. Section from site of injection at margin. Note the new blood vessel and the preponderance of large mononuclear leukocytes in the inflammatory reaction. $\times 1200$.

FIG. 4. Cornea of same rabbit 2 weeks after double injection of tubercle bacilli. Section from site of injection at center. Note persisting preponderance of polymorphonuclear leukocytes and absence of blood vessels. $\times 1200$.



3

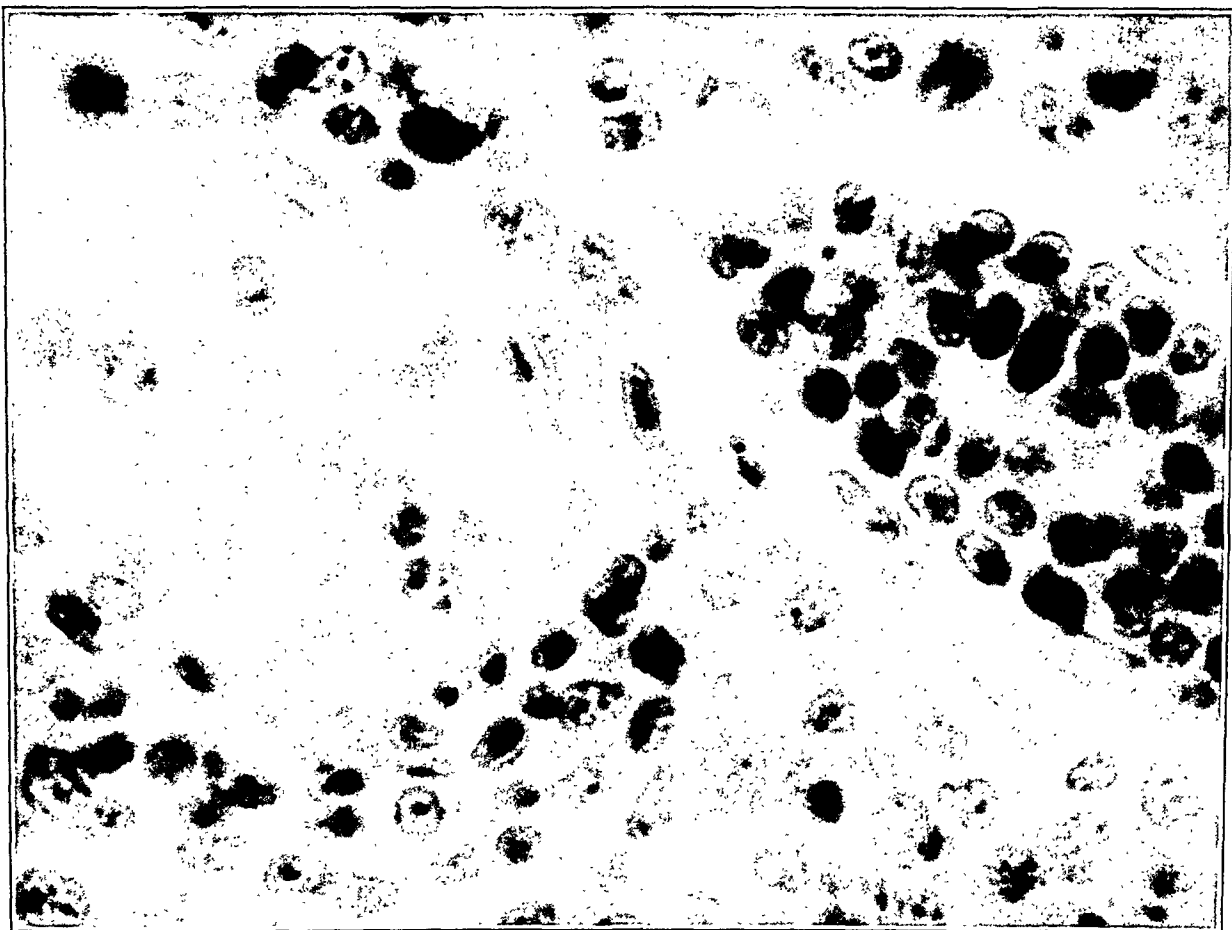


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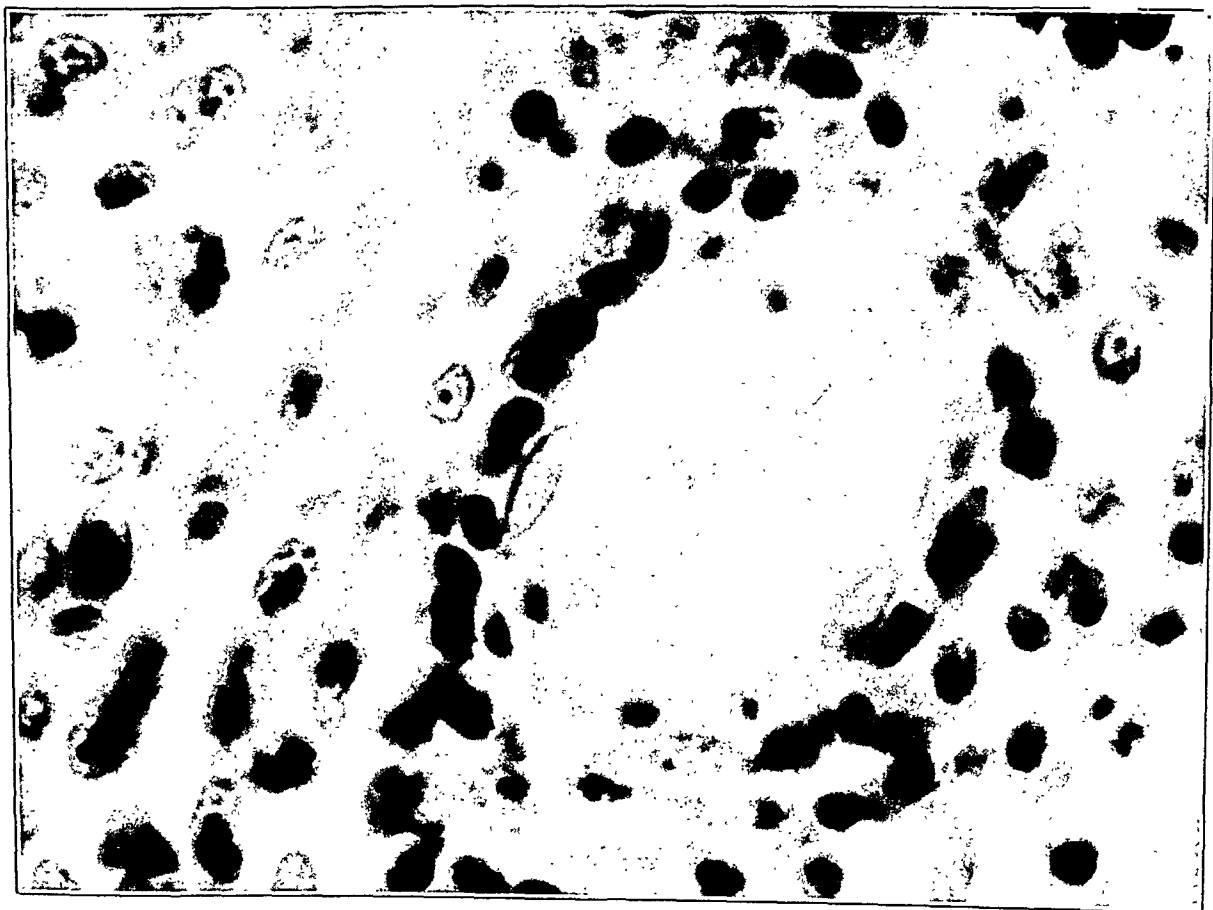
PLATE 56

FIG. 5. Cornea of rabbit 48 days after double injection of tubercle bacilli. Section from site of injection at margin. Note new blood vessels and accumulations of large mononuclear leukocytes around and in new vessels. Note also gradual change in character of large mononuclears outside of perivascular collar of cells. $\times 1200$.

FIG. 6. Cornea of same rabbit 48 days after double injection of tubercle bacilli. Section from site of injection at center. Note new blood vessel and collar of hyperchromatic large mononuclear leukocytes. Very few polymorphonuclear leukocytes remain. Note changing character of large mononuclears outside of perivascular collar of cells. $\times 1200$.



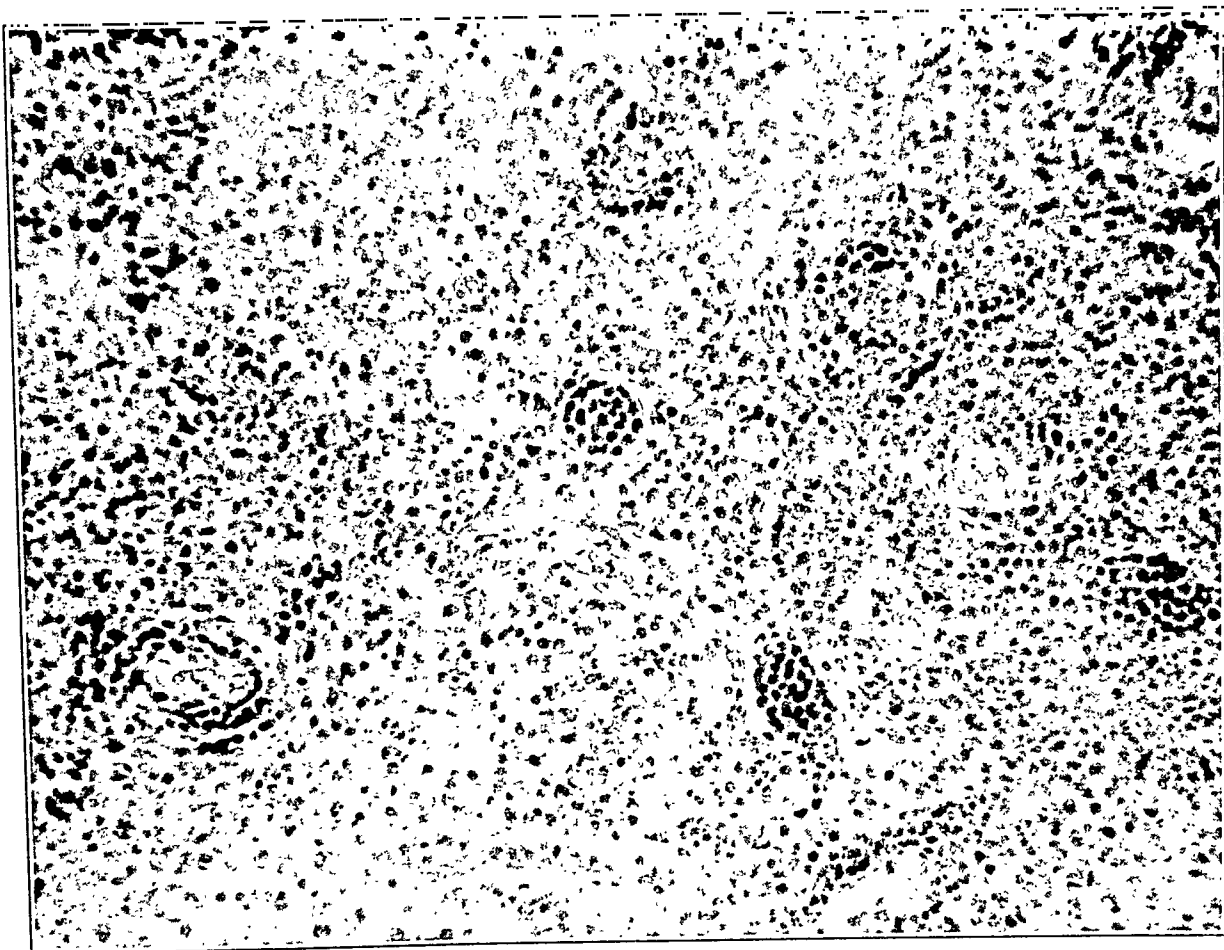
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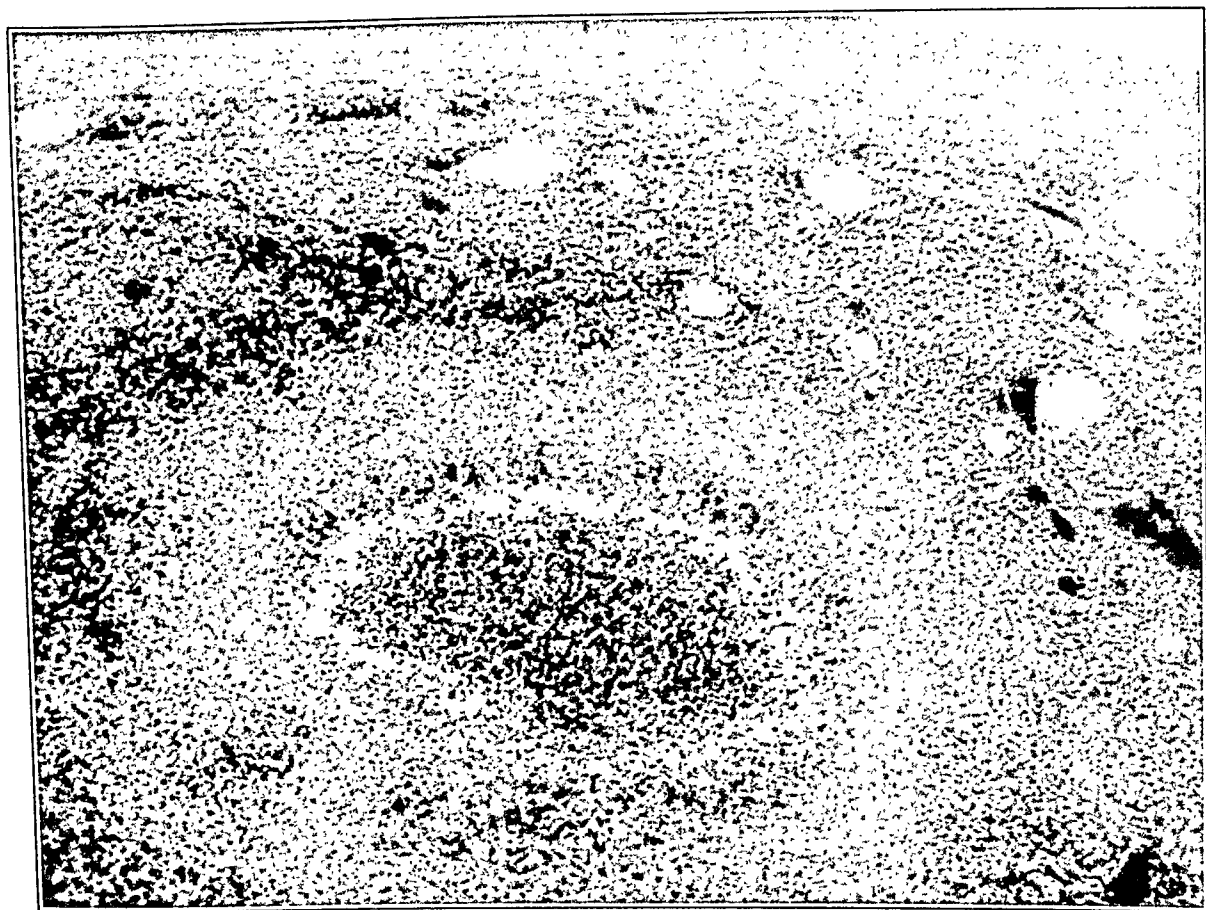
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PLATE 57

- FIG. 7. Cornea of rabbit 48 days after double injection of tubercle bacilli. Section from site of injection at margin. Note accumulations of large mononuclear leukocytes in and around new blood vessels and in tissue spaces. Note change from dense hyperchromatic nuclei in and near vessels to vesicular nuclei in surrounding tissue. $\times 260$.
- FIG. 8. Cornea of same rabbit 48 days after double injection of tubercle bacilli. This section also from site of injection at margin. Note new blood vessels and cells with hyperchromatic nuclei in association with them and note spreading of these cells away from vessel walls into surrounding tissue. Note change of cells to epithelioid type and region of necrosis (typical tubercle). $\times 50$.



7



8

PLATE 58

FIGS. 9-13. New blood vessels in rabbit cornea 1 month after injection of tubercle bacilli. Note migration figures (outlined) of large mononuclear leukocytes in course of transit from inside to outside of vessel. $\times 600$.

FIG. 14. New blood vessel in same rabbit cornea (outlined). Note large number of large mononuclear leukocytes in lumen. $\times 600$.



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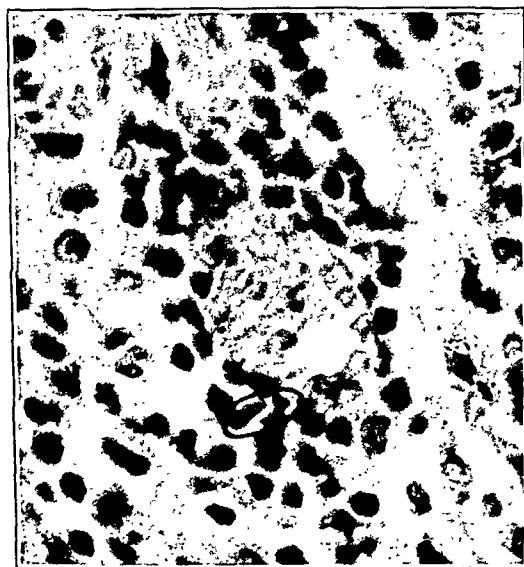
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14

THE HISTOLOGICAL CHANGES IN THE THYROID GLAND OF THE WHITE RAT EXPOSED TO COLD*

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In a series of articles beginning in 1916 and ending in 1928 Cramer and Ludford,¹⁻⁴ working in London, recorded in rats and mice exposed to cold increased vascularity of the thyroid gland, cellular hypertrophy with enlargement of mitochondria and Golgi apparatus, altered staining properties of the colloid followed by a decline in its amount and, eventually, death of the epithelium. This was interpreted as meaning increased secretory activity of the gland induced by the metabolic strain. Conversely, at warm temperatures the colloid increased in amount, the cells became flatter and the injection diminished. The importance of these observations lies (1) in the rôle of the thyroid in heat regulation thus suggested, (2) in their bearing on the long known seasonal incidence of endemic goiter, and (3) in the promise that they give of providing us with a means of modifying thyroid structure by an easily manipulated experimental condition that can safely be regarded as demanding, through the normal channels, augmented secretion. It is chiefly with this last interest in mind that the present study has been directed toward the resolution of the discrepancies existing between the work of Cramer and certain of his successors, discrepancies which have their ever perplexing counterparts wherever the effect of stimulated metabolism on the histology of the thyroid has been sought.

In Cramer's published reports experimental details are given sketchily, so that it is often difficult to get an exact picture of the environment and of the degree and time of histological change. Some effects were observed in "a mouse exposed to cold for three hours," "a mouse kept four hours in a laboratory with an open window during cool weather in March," and "a rat epilated and kept in a glass jar on a cold winter day." More extreme changes are figured in 2 rats kept in a cold room at 4° C for 10 and 14 days respectively. Ludford⁴ obtained the most extreme changes in rats epilated and

* Received for publication January 13, 1933.

kept 24 hours in an ice-chest. The effect of heat ($30-35^{\circ}\text{C}$) was apparently visible in 6 hours in the mouse.

Mills,⁵ working in South Dakota in 1918, found quite regularly, as compared to the preliminary biopsy, increased cell height, vacuolation of the colloid and a decline in its amount in 16 rabbits and 4 guinea pigs exposed in a cold room or outdoors at -5 to $+10^{\circ}\text{C}$ for from 3 to 30 days. In 28 rabbits, 2 cats and 3 guinea pigs exposed in a well ventilated hot-box at 27 to 37°C all but 4 animals showed decreased height of epithelium and increase in the amount of non-vacuolated colloid.

Hart,⁶ in Berlin in 1922, reported on gray house mice kept at 32 to 40°C until they died and at 4 to 7° for a time not stated. The pictures given for high temperatures are for 1 animal kept 38 days and another "several days." The epithelium is cubical, the colloid reduced, the follicles folded and empty. At low temperatures the cells are cubical, the colloid abundant. Verbally, Hart agrees with Cramer, Ludford and Mills that the thyroid is more "active" at low temperatures; actually, as Wegelin⁷ points out, his findings are contrary to theirs as he uses accumulation instead of reduction of colloid to measure activity.

Bergfeld⁸ in 1930, working in Freiburg, found that the thyroids of 3 male rats and 1 female, kept in the darkness in a cold cellar at from $+2$ to -3°C for 4 to 6 weeks, showed cellular hypertrophy and loss of colloid, while those of 3 males kept 6 weeks in a cellar illuminated by unfiltered daylight were normal. Again, in animals living 6 weeks in thermostats at 29°C , 3 in darkness had hypertrophy of the thyroid epithelium, while 3 in the light did not. Bergfeld concludes accordingly that darkening acts without reference to temperature to cause changes that indicate an "increased function," and that temperature alone is without influence. While the pictures of these Freiburg animals reveal differences too slight to convince, Bergfeld later,⁹ working at Davos, Switzerland, adduced better evidence that the thyroids of rats deprived of ultraviolet light for 21 to 63 days were hypertrophied and poor in colloid. Rosenkranz¹⁰ in Davos, in a small number of rabbits examined after 4 weeks, and Turner and Benedict^{11,12} in New York, in a large number of Barred Rock chicks observed for from 55 to 105 days, confirmed Bergfeld's account of the effects of the absence of ultraviolet light, so that despite the negative findings of von Fellenberg¹³ the factor of illumination must be taken

seriously. As neither Bergfeld nor Turner and his coworkers were able to detect any increase in the basal metabolism of the darkened animals, the mechanism remains for the present quite obscure.

NOTE: Space does not permit citation of the extensive scattered literature on the seasonal incidence of endemic goiter, nor of such studies of the geographical distribution of thyroid diseases as have a bearing on the effects of environmental temperature. Although of great interest, they are non-analytical in nature and in no case permit decision as to the relative importance of light, temperature and food supply. In the absence of any complete review of this subject the reader may refer to the articles in the monograph by Kendall.¹⁴

METHODS

Albino rats bred from a single stock were used. They were housed in quarters with a temperature range of 20 to 30° C, illuminated by diffuse daylight with some sunshine passing through windowglass to the cages. They were fed the standard Steenbock diet ¹⁵ consisting of:

Yellow corn	76.0
Linseed oil meal	16.0
Crude casein	5.0
Ground alfalfa	2.0
Sodium chloride	0.5
Sodium carbonate	0.5
Milk and water <i>ad lib.</i>	

Young animals from 43 to 105 days of age, weighing from 73 to 260 gm., paired for weight and sex and chosen in the great majority of cases from the same litter, were selected for experiment and as controls. Separate controls were run for each experimental period, as a single sampling of the stock does not suffice where temperature, light and food of necessity vary, and any or all may be of influence.

Most of the experiments were run in complete darkness. Exposure to cold was secured in a large, dark, refrigerated room in the hospital morgue, where the average temperature during the several experimental periods varied from -4 to +2° C. Ventilation, secured only by frequent openings of the door of the chamber, was sufficient to keep the oxygen and carbon dioxide content of the air, with animals present, not far from that of the ordinary dwelling room (O₂ - 20.76%, CO₂ - 0.1%). The occasional introduction of a corpse painted with iodine for surgery was unfortunately unavoidable, and although during several experimental periods this was known not to have occurred, the iodine content of the atmosphere must have been

high at times. While this may have contributed to the variability of the results it could not, as will appear subsequently, have materially altered the essential conclusions. The animals were placed 2 or 3 in a cage with beds of shavings and fed the stock diet with milk, or the following modification with water:

Yellow corn	62.0
Linseed oil meal	13.0
Crude casein	4.0
Ground alfalfa	2.0
Whole milk powder	18.0
Sodium chloride	0.5
Calcium carbonate	0.5
Water as desired	

The fact that the refrigeration of this chamber was controlled by a central plant operated to supply the entire building made it impossible to regulate the temperature locally. During certain experimental periods the fluids supplied the animals were observed not to be frozen at the time of feeding, while at other times freezing occurred and the chipping of the ice by the animals was insufficient to secure the usual intake. As the water consumption of a chilled rat is small and the chamber damp, no serious systemic effect of the restriction was likely, nor was any observed. The influence on iodine intake will be discussed later.

The controls were kept in a large, well ventilated photographer's dark room at average temperatures for the several periods of 20 to 31° C, fluctuating, of course, with the season. They were caged, cleaned, fed and watered precisely the same as their brothers and sisters in the cold.

At the completion of the run the animals were weighed and killed by a blow on the head. The thyroids were removed as rapidly as possible without sacrificing tissue and placed directly in Zenker's or formol-Zenker's fluid for fixation. Shortly thereafter the glands were weighed on an analytical balance, the difference between such weights and the fresh weights being insignificant. Under any circumstances the weights of the glands are to be regarded as approximate only, variations in drying and adherence of bits of surrounding muscle and fat precluding pretensions to refinement. The tissues were then dehydrated and embedded in paraffin. Sections were made serially through the entirety of both lobes of the first 110 glands

studied, and thereafter through the center of the larger lobe. Staining was with hematoxylin-eosin.

In interpreting slides arbitrary grades were assigned to the height of the epithelium, size and colloid content of the follicles, vacuolation of the colloid and vascular injection, without knowing the source of the tissue. Estimations were reached from serial studies of the entire thyroid mass when the sections were available, and later from central sections, which are, on the whole, representative. Vacuolation was recorded as absent or present in grades of 1, 2 and 3; injection as minimal (0) and as grades 1 and 2. These two estimations are difficult and are in addition subject to error introduced by variations in fixation and in vascular engorgement of the region at the time of death, and are hence to be regarded as the merest approximations. Cellular size was recorded as flat epithelium 1, cuboidal 2, low columnar 3, and high columnar 4. Amount of colloid was recorded as little or none, 0 — 1; small follicles filled with colloid, 2; medium follicles filled with colloid, 3; large follicles filled with colloid, 4. Occasional occurrence only was indicated by placing the figure in parenthesis. No attempt was made to assign any degree of follicular filling between that of completeness and that of practical depletion, in certain cases large plicated follicles that had obviously suffered a loss of content being passed as full. This error, which was infrequent, tends toward underestimation of the colloid loss. While such designations as these poorly depict the variety of living tissue, they are useful in reviewing data and in making tabular presentations of anatomical material. Moreover, with experience, they can be made with fair accuracy.

THE CONTROL THYROID GLAND

The thyroid glands of 64 control animals kept in the dark for from 5 to 25 days, together with those of 23 others receiving ordinary illumination, may be conveniently described together as the baseline for the present work (see the next section). The gland is a bilateral structure usually fairly symmetrical, but showing at times every grade of asymmetry, not infrequently to the absence of a lobe. The isthmus is usually only a connective tissue strand, but may be parenchymatous. The average weight is 12.7 mg. per 100 gm. body weight, varying from 4.1 to 25.4 with only 5 exceeding 20 mg. per 100 gm. It

may be so small as not to be discovered by the ordinary dissection. Microscopically the two lobes correspond. The epithelium is, as a rule, cuboidal to low columnar and the amount of colloid moderate (graded 2, 3 to 3) (Figs. 2 and 4). The follicles may be large or small throughout a gland, with a regular distribution of larger, well filled follicles lined with flatter epithelium lying superficially. Centrally the follicles are smaller, the cells a little higher, and foci of diminished colloid content, marked often by hypertrophy, appear in 17 per cent of the controls. While of varying size these foci are usually small, being only once large enough to dominate the gland. It is of interest that of the 15 control glands that have such areas of colloid reduction 11 occurred, for no known reason, in the first 32 studied during a period from March to May, 1931, the phenomenon being rare since. Adenomas have never been seen.

THE INFLUENCE OF LIGHT

A brief preliminary comparison was made at 20° C between the effects of darkness and diffuse daylight entering the room through a glass window. Five pairs of litter-mates were kept for 10 days, one group in the dark room and the other in a well lighted laboratory. Table I, Part 1, shows that there was no difference between them. Table I, Part 2, includes these, and in addition 22 others of approximately the same age but not strict litter-mate controls, that were examined between March 11 and May 28, 1931. The height of the epithelium, degree of colloid reduction and gland weights were the same. Inspection of controls kept for periods of 15 to 25 days in the dark at 25° to 31°, as for instance those given in Table III, showed no tendency to reduction of colloid or hypertrophy of epithelium. Darkness alone, then, whatever its effect may be with stricter comparisons over longer periods of time, does not, within 25 days, produce striking hypertrophy and colloid loss. This is not intended to contravert the evidence concerning the importance of ultraviolet rays for thyroid function, but merely to emphasize that the striking effects to be detailed in animals exposed to cold cannot be attributed to exclusion of light.

THE EFFECT OF EXPOSURE TO COLD

A group of preliminary experiments of animals exposed to cold for from 6 to 24 hours showing nothing in particular, attention was directed to protracted experiments lasting from 10 to 25 days. Only such will be reported here.

The mortality was surprisingly low, especially if animals weighing over 80 gm. were used. A series of comparisons of 17 animals kept at

TABLE I

Comparison of the Thyroid Glands of Rats Kept 10 Days in Light and Darkness, March 11-May 28, 1931

Experiment	No. of animals	Amount of colloid					Height of epithelium					Average wt. of thyroid mg. 100 gm.			
		Most foci absent	0-1	1, 2	2, 3	3, 4	3, 4	3	2, 3	2	1, 2				
<i>Part 1. Litter-Mate Controls</i>															
Light at 20° C	5	0	1	4	0	0	1	4	0	1	2	2	0	0	10.2
Dark at 20° C	5	0	2	3	0	0	3	2	0	2	1	2	0	0	10.2
<i>Part 2. Litter-Mate Controls Plus Others of Same Age</i>															
Light at 20-21° C	12	0	4	8	0	3	2	7	0	2	5	5	0	0	12.9
Dark at 20-24° C	20	1	6	13	0	3	10	4	2	6	4	6	4	0	14.2

Key: The first series of columns under "amount of colloid" designates the occurrence of areas of little or no colloid (0-1) throughout the gland, in foci or absent. The second series of columns gives the distribution of various grades of follicular colloid content predominating in such glands as have more than traces: 2 = small follicles filled with colloid; 3 = medium follicles filled with colloid; 4 = large follicles filled with colloid. Such conjoint expressions as 0-1, 2 indicate that both degrees are found in abundance. Under "height of epithelium" 4 indicates high columnar; 3 = low columnar; 2 = cuboidal, and 1 = flat. Conjoint expressions as 3, 4 indicate that both types are present in large numbers.

0° and -3° C for 10 days with their controls showed no decline in the rectal temperature. The animals exposed to cold only rarely failed to gain weight, but such gains, as a rule, amounted to little more than half that of the controls.

Ensuring an abundance of food made little difference. Table II presents evidence that the amount of weight gained is in no way correlated with the degree of change described in the thyroid gland.

Age, within the limits studied, sex and stage of the oestrus cycle were without discernible influence on the structure of the gland.

Grossly the glands of the animals exposed to cold were often turgid, red, and at times massively enlarged. Of 72 rats exposed to temperatures averaging from 0 to -4° for from 10 to 25 days in 8 series between March 1931 and March 1932, 32 showed reduction of colloid to traces through the greater bulk of the gland, 29 reduction in foci of various sizes, and in only 11 no such reaction. The epithelium was low to high columnar in 59, and in one case only was low cu-

TABLE II

Comparison of the Effects of Maximal and Minimal Weight Gain on the Thyroid Glands of Rats Exposed to Cold. Comparative Animals of Similar Age and Sex, Run at the Same Time, Are Selected from Groups in Table III

Experiment	No. of animals	Amount of colloid					Height of epithelium					Average wt. of thyroid mg. 100 gm.			
		Most foci absent													
			0-1	2	3	4	3	4	5	6	7				
Maximal (average + 32.2 gm.)	13	7	6	0	4	2	2	0	0	13	0	0	0	0	19.7
Minimal (average + 7.4 gm.)	13	5	6	2	3	1	3	1	0	9	1	3	0	0	19.9

For key see Table I.

boidal to flat. Sixty-two of these animals had 49 strict controls, the great majority litter-mates (Table III). Of the experimental group 26 showed preponderating reduction of colloid to traces, of the control rats 1; 25 reduction in foci, of the control 5; no such reduction in 11, in the control 43. High columnar epithelium prevailed in 49 of the 62 experimental animals and in 5 of the 49 controls (Figs. 1, 3 and 5).

The average weight of the gland per 100 gm. body weight was 19 mg. in the experimental, 13 mg. in the controls. In 7 cases the weight of the gland in the experimental animals exceeded the maximum of the control glands, including such values as 62.5, 50.8 and 38.2 mg. per 100 gm. Out of 57 strict comparisons the gland of the animal exposed to cold was 1 mg. or more per 100 gm. body weight heavier than the control in 42.

TABLE III

Comparison of the Thyroid Glands of Animals Kept in the Cold with Those at Normal Room Temperature

Experiment	No. of animals	Amount of colloid					Height of epithelium					Average wt. of thyroid mg. 100 gm.			
		Most foci absent	0-1, 2	2, 3	3	3, 4	3, 4	3	2, 3	2	1, 2				
Four Groups April 3 to May 29, 1931															
10 days 0 to -3°C	17	11	5	1	2	1	2	1	0	15	1	1	0	0	17.4
10 days 20 to 24°C	15	1	4	10	0	3	7	2	2	4	3	4	4	0	14.8
June 12 to July 2, 1931															
20 days -3°C	7	7	0	0	0	0	0	0	0	7	0	0	0	0	34.8
20 days 31°C	6	0	0	6	0	0	2	2	2	0	1	4	0	1	12.3
August 24 to September 18, 1931															
25 days -1°C	7	6	0	1	0	0	0	1	0	6	1	0	0	0	19.9
25 days 28°C	7	0	0	7	0	1	1	4	1	0	3	1	1	2	11.8
October 9 to November 2, 1931															
20 days -2°C	6	1	3	2	2	0	0	1	2	5	1	0	0	0	17.8
20 days 24°C	4	0	1	3	0	1	0	2	1	1	0	2	0	1	10.9
December 11 to 28, 1931															
17 days -3°C	13	1	7	5	2	4	5	1	0	6	2	3	1	1	16.5
17 days 25°C	7	0	0	7	0	0	7	0	0	0	0	2	5	0	12.3
February 19 to March 11, 1932 — Modified Diet with Water															
21 days -4°C	12	0	10	2	6	0	5	0	1	10	1	1	0	0	14.0
21 days 24°C	10	0	0	10	0	0	5	5	0	0	6	4	0	0	12.4
All Groups with Precise Control															
10-25 da. 0 to -4°C	62	26	25	11	12	5	12	4	3	49	6	5	1	1	19.0
10-25 days 20- 31°C	49	1	5	43	0	5	22	15	6	5	13	17	10	4	13.0
Total March 11, 1931, to March 11, 1932															
10-25 da. 0 to -4°C	72	32	29	11	15	5	13	4	3	59	6	5	1	1	18.6
Total 20- 31°C	87	1	14	72	0	10	40	26	10	12	20	30	19	6	12.7

The total group adds to those already given such animals as were submitted to similar conditions in other connections and had accordingly no precise controls. Those kept at 20 to 31°C include 23 kept in the light and 2 kept 5 days in the dark. Except when indicated, the stock diet with milk was used.

For key see Table I.

This process of hypertrophy and colloid loss was most extreme centrally, but in the more severe instances the poles were involved. When the larger peripheral follicles were affected the walls became irregular and plicated, a bulging capillary tunneling each epithelial crest (Figs. 1 and 5). In general, the vacuolation of the colloid was striking, and remnants in nearly empty follicles became granular in character. Mitotic figures were often present. In one series they were counted according to the technique of Gray and Loeb¹⁶ of enumerating those seen in every tenth 10 micron section. Values of 35.4, 158, 223, 7.9, 39.1, 48 and 1.3 per mg. of gland were found, as compared to none in the 6 controls. The glands were commonly more vascular microscopically, although this is in many cases not easy to determine. In certain extremes the follicles are reduced to solid epithelial columns separated by dilated sinuses of blood (Fig. 5). Necrosis or desquamation of epithelium, described by Cramer, was notably absent save in two animals examined several hours after death, although collapsed injured follicles, not unlike those pictured by this author, may be seen at the periphery of any gland, whether the animal was exposed to cold or not, and due, presumably, to manipulation.

While the character of the changes was consistent throughout, the degree was not. In the experiments performed in the spring and summer of 1931 the hypertrophy and colloid loss frequently dominated the gland; in those performed in the late fall and winter of 1931-32 such changes, although consistent, were focal for the most part. This variation was especially striking with regard to weight. A 20 day experiment from June 12 to July 2, 1931, produced glands averaging 34.8 mg. per 100 gm. body weight for 7 animals, as compared to a control of 12.3; while a 21 day experiment performed between February 19 and March 11, 1932, produced glands averaging 14 mg. in 12 animals, as compared to a control of 12.4. As the controls for these two groups were microscopically similar, variation in the stock does not account for the differences. Such irregular results, unfortunately common enough in work on the thyroid, are not easy to explain but, as will appear later, may be due to uncontrolled variations in the iodine content of the diet or of the atmosphere.

THE EFFECT OF IODINE ON THE CHANGES INDUCED

Table IV gives the results on three comparative series, running from 15 to 21 days, comprising in all 50 animals, of which half received 10 y* of iodine daily in the form of potassium iodide dissolved in physiological saline and injected subcutaneously. The effect was to reduce materially the incidence of hypertrophy and colloid evacuation. Thus only 6 animals showed areas of marked colloid diminu-

TABLE IV

Showing the Influence of Iodine, 10 y Daily, on the Response of the Thyroid to Cold

Experiment	No. of animals	Amount of colloid					Height of epithelium					Average wt. of thyroid mg. 100 gm.
		Most foci absent	0-1, 2	2, 3	3	3, 4	3, 4	3	2, 3	2	1, 2	
July 5 to 20, 1931 — Stock Diet with Milk												
15 days at 0° C with iodine	6	1 1 4	0 1 3 1 0				1 3 2 0 0				8.5	
15 days at 0° C no iodine	5	5 0 0	0 0 0 0 0				5 0 0 0 0				16.3	
December 11 to 28, 1931 — Stock Diet with Milk												
17 days at -3° C with iodine	9	0 1 8	1 1 7 0 0				0 1 4 1 3				10.2	
17 days at -3° C no iodine	8	1 5 2	1 3 3 0 0				4 2 1 1 0				16.3	
February 19 to March 11, 1932 — Modified Diet with Water												
21 days at -4° C with iodine	10	0 3 7	0 1 7 1 1				0 5 5 0 0				11.7	
21 days at -4° C no iodine	12	0 10 2	6 0 5 0 1				10 1 1 0 0				14.0	
All Groups												
Cold + iodine	25	1 5 19	1 3 17 2 1				1 9 11 1 3				9.8	
Cold only	25	6 15 4	7 3 8 0 1				19 3 2 1 0				15.2	

For key see Table I.

* y = gamma 0.001 mg.

tion as compared to 21 receiving no iodine; 1 had high columnar epithelium as compared to 19 receiving no iodine; the average weight of the glands was 9.8 mg. per 100 gm. body weight as compared to 15.2.

The amount of iodine was much less than the daily 0.5 and 2 mg. KI observed by Rabinovitch¹⁷ to increase mitotic proliferation in the rat when given for 5 to 10 days; indeed the mitotic proliferation induced by cold was suppressed. Thus, in one series 5 animals receiving no iodine had 8.2, 57.8, 10.9, 4.9 and 6.4 mitoses per mg. of gland, as compared to 0, 0, 0, 0, 0.9 and 14.4 for the 6 animals receiving iodine.

Two groups were compared, one kept in the cold and the other at room temperature, both receiving 10 y of iodine daily (not given in the tables). The effect of cold on the epithelium and colloid content was very nearly completely obscured, although the glands of the 7 animals kept in the cold averaged somewhat heavier, 13.6 as compared to 8.5 mg. per 100 gm. body weight.

The effect of iodine in partially or completely abolishing the transformation effected by cold may very well provide an explanation for the discrepancies between the work of Cramer and Mills on the one hand and those of Hart and Bergfeld on the other, by virtue of the inevitable variation in the iodine content of natural diets and the likelihood of iodine permeating the air of laboratories.

THE SIGNIFICANCE OF HYPERTROPHY AND COLLOID LOSS

The contentions of Cramer and Mills being herein largely borne out under circumstances in which the animal's metabolism is extraordinarily increased (approximately 150 per cent according to the careful studies of Benedict and MacLeod¹⁸), it is natural to conclude forthwith that the changes induced represent increased delivery of thyroid hormone to the blood stream. While the writer is inclined to this view, to state it so baldly is to ignore the subtle confusion which pervades the entire subject of the meaning of the hypertrophy of the thyroid cell. Some general considerations are hence necessary.

The structure of the anatomical unit of the thyroid as a closed cellular vesicle holding a thyroglobulin-containing colloid necessitates the view that this colloid represents the secretion of the thyroid gland over and above that being delivered to the tissues. This re-

mains true whether colloid formation is obligatory as a phase in the synthesis of the hormone, or whether, following Bensley,¹⁹ it is an alternative to direct export of secretion from cell to circulation. For in either case, in an extremity, colloid is withdrawn.

Loss of colloid may therefore occur either as the result of an impairment of its formation or because of unusual excretion. Below is a schematic presentation of the various factors which may operate in either case. Although given abstractly, nearly every point has entered into someone's theory.

A. Impaired formation of colloid:

1. Because the ingredients of the colloid are unavailable.
2. Because the cells themselves suffer from some specific inability to form the colloid.
3. Because some extrinsic mechanism stimulating colloid formation fails.
4. Because some extrinsic mechanism inhibiting colloid formation overacts.

B. Increased export of the substance of the colloid or of a derived substance:

1. Because the constitution of the colloid itself makes it impossible of retention (see A, 1 and 2).
2. Because of increased stimulation by an extrinsic mechanism acting either (*a*) in accord with tissue needs, or (*b*) independent of them.
3. Because of diminished inhibition by an extrinsic mechanism acting either (*a*) in accord with tissue needs, or (*b*) independent of them.
4. Because of increased release of indigenous origin, as in neoplasms.

Now it is unfortunate that as yet no distinction can be made between A, impaired formation, and B, augmented excretion on cytological grounds. Hypertrophy and hyperplasia of the epithelium and increased vascularization accompany the process of colloid solution and loss in both instances. Exophthalmic goiter in man and the hyperthyroidism induced experimentally by anterior pituitary preparations at once suggest themselves as instances in which the excretion of the hormone is excessive; while in endemic goiter for all the intense hypertrophy and marked reduction of colloid there are no signs that the organism is being flooded with a recognizable thyroid hormone. The most modern iodine restriction studies furthermore present excellent evidence that when this essential element of the secretion is lacking, hypertrophy accompanies the colloid loss. Thus Levine²⁰ and Remington²¹ in South Carolina have recently reported diffusely hyperplastic goiters, four times the normal size, occurring in white rats kept 35 days on a diet containing 15 parts of iodine per

billion, the stock diet having an iodine content of 42 to 72 parts per billion. This confirms the earlier work of McClendon and Williams,²² Hayden, Wenner and Rucker,²³ Drennan, Malcolm and Cox,²⁴ and Krauss and Monroe,²⁵ and negates that of Hellwig²⁶ and Jackson and P'an,²⁷ who, unfortunately, have not provided actual analyses of the iodine-poor diets with which they worked.

A parallel to this discrepancy between hypertrophy and basal metabolism exists following administration of iodine to animals with diffusely hyperplastic goiters. There is always a recession of hypertrophy to some extent, however patchy, together with an accumulation of colloid, but the effect on the basal metabolism varies with the state of the animal. In human hyperthyroidism after the use of iodine there is a decline in the basal metabolism, often incomplete and transient but definite and important. Likewise, in the hyperthyroidism provoked in the guinea pig by those anterior pituitary preparations recently developed by Loeb and his coworkers,²⁸⁻³⁰ Aron,³¹ Schockaert,³² Krogh, Lindberg and Okkels,³³ and others, iodine suppresses the greater part of the rise in the metabolism (Siebert and Thurston⁴³). However, in the endemic goiter in the rabbit described by Chesney, Webster and Clawson^{38,44} in which the metabolism is primarily low, iodine induces a sharp, intense and often fatal hyperthyroidism,^{45,46} which takes place apparently while the cellular hypertrophy is receding and colloid is accumulating. This effect was confirmed by Marine (cited by Webster and Chesney⁴⁵) who, curiously enough, had with Lenhart observed it years ago in goitrous dogs and made little of it.⁴⁸ It is presumably similar to the toxic reactions that halted the early iodine therapy of endemic goiter in man,⁵⁰ and which are now, with a few often doubtful exceptions, rarely seen in America in any precise and unmistakable form. Rienhoff⁴⁷ was quite unable to produce them by deliberately giving iodine to patients with nodular goiters. It may well be that these

NOTE: For many illustrations of hyperplastic thyroids occurring in endemic goiter, Marine⁴⁴ may be consulted; for a discussion of the disparity between the histological findings of intense activity of the thyroid and a normal general state of health in the dog, Carlson and Woelfel²⁵; for the occurrence of the cretin, hairless pig in such stock, Hart and Steenbock²² and Smith²⁷; for a recent determination of low basal metabolism in the rabbit with diffusely hyperplastic endemic goiter, Webster, Clawson and Chesney³⁸; for discussion of normal clinical states and normal basal metabolisms in human adolescents with diffusely hyperplastic goiters, Gold and Orator,²⁹ Wegelin,⁷ de Quervain,⁴² and de Quervain and Pedotti⁴¹; for the appearance of human cretinism in the presence of abundant hyperplastic thyroid tissue, Wydler.⁴²

inconsistencies between present clinical experience and the Hopkins experiments are more apparent than real, and have as their explanation the present rarity in man, especially in America, of the massive diffusely or abundantly hyperplastic endemic goiter, which forms the anatomical substrate for the experimental iodine hyperthyroidism. No student has as yet been able to reconcile in a comprehensive theory these two apparently opposing phenomena — the stimulation of basal metabolism by iodine in certain endemic goiters, and the suppression of elevated metabolism in human exophthalmic goiter and in animal hyperthyroidism due to pituitary injection. It is sufficient for the present to indicate that both indubitably occur and that as with hypertrophy and evacuation of colloid, recession of cellular enlargement and accumulation of colloid cannot measure precisely the amount of thyroxin being delivered to the organism.

To Marine,^{34, 48, 49, 50} speaking from his experience with the resolution of simple goiter by iodine, we are indebted for the forcible presentation of the idea that hypertrophy expresses a relationship between supply of hormone ingredients (stated in terms of iodine) and excretion of hormone, rather than simply augmented excretion of hormone from the gland. Unfortunately, however, this author's use of two terms, "work hypertrophy" and "compensatory hypertrophy," in connection with the reaction to iodine insufficiency, has contributed confusion: the former term because, while conveying properly enough the impression of physiological strain that enlarging and multiplying cells must always give, implies a physiologically significant hypersecretion, of which there is no evidence; while "compensatory hypertrophy," when the gland substance has not been actually reduced, substitutes a vague teleology for specific anatomical meaning which, in the end, must be found for so regular, common and clear-cut a phenomenon. Plummer⁵¹ has, with characteristic subtlety, suggested that the hypertrophied thyroid cell may always be excreting, under stimulation, an excessive amount of fluid menstruum which varies, however, in its thyroxin content from the large amount present in exophthalmic goiter to virtual absence in certain cretins.

Tentatively, then, for the purpose of the interpretation of the work herein presented, a view not far from those of Marine and Plummer will be adopted to the effect that hypertrophy with declining colloid content represents preponderating excretion of the hormone from

the gland, whether the amount excreted be great or small. Such hypertrophy may result from either insufficient material to form the hormone needed or from excessive export of the substance formed. The distinction between these two can only be made by: (a) demonstrating the absence or unavailability of some known ingredient of the secretion, or (b) recognizing the effects of thyroxin operating in the organism to a greater or less degree, or in some future time measuring the thyroxin content of the blood.

THE INTERPRETATION OF THE EFFECTS OF COLD

The significance of the hypertrophy and colloid loss regularly observed in the thyroid gland when rats are exposed to cold for 10 days or more can only be established when it is known that the essential ingredients of the colloid are being adequately supplied and that there is an increased demand for the hormone under these conditions.

There are no good grounds for supposing that the animals are deprived of any essential ingredient of the hormone. They eat more dry food than their controls, the process occurs when the milk intake is not seriously limited by freezing (7 animals, 25 days — August 24 to September 18, 1931; 5 animals — April 15 to 26, 1931), or when dried milk is used and the fluids supplied as water (Diet 2, 12 animals 21 days — February 19 to March 11, 1932); so that it is unlikely that any known food constituent is absolutely restricted in the experimental animals as compared to the controls, although it is theoretically possible that some essential substance might be used in large amounts by the active muscles and hence deviated from the gland. As for iodine, any loss due to freezing of water (using McClendon's estimate of 0.67 part in one billion of 68th Street crib Lake Michigan water) is made up many times by the ingestion of additional food that must contain much more than the 15 parts per billion found goitrogenic for the rat by Levine and Remington or the 10 parts per billion in McClendon's most iodine-poor regimen.

That the capacity of the cells to form the hormone was not impaired may be judged from the normal cytology, and from the fact that iodine so completely prevented the change.

The heightened tissue activity induced by exposure to cold includes increased muscle work plus any such direct stimulation of basal metabolic processes as may take place, these two phenomena

occurring in completely unknown proportions at this intensity of cold. Benedict and McLeod, after carefully studying activity records of white rats at 21 and 28° C, concluded that differences in heat production could not be accounted for by the differences in the recordable movements of the animals. Furthermore, rats kept at 21° for 24 hours have a slightly higher basal metabolism, measured at 28°, than when kept for 24 hours at 28° (4.5 per cent in all but 1 of 11 animals). The quantitative importance of this suggested increase in basal metabolism induced by cold at such temperatures as alter thyroid structure and the actual rôle of the thyroid in its production remain undetermined.

It is natural enough to relate thyroxin utilization to the augmented total metabolism induced by cold. Cramer, Ludford and Mills have done this and are in accord with one of the most popular conceptions of the action of the thyroid hormone, that of Plummer, to wit: "Thyroxin is a catalyst hastening the rate of formation of a quantum of potential energy available for transformation on excitation of the cell." The great difficulty here rests in the uncertainty concerning the place of thyroxin in the metabolism of active muscle. Boothby, Buckley and Wilhelmj⁵² have recently, in the dog, found the calorogenic action of thyroxin greater during a period of work in a treadmill than before the work was started. If thyroxin were used up by the active muscles its calorogenic action should have been less during work. While, as recognized by these workers, this experiment would have been more conclusive if performed on thyroidectomized animals, it does create at present a substantial obstacle to the relating of the function of thyroxin in a simple quantitative way to muscular oxidative activity in general. On the other hand, several recent physiological studies⁵³⁻⁵⁶ have concurred in the finding that the neuromuscular system of the heart removed from central nervous control responds to thyroxin by increased contraction. Of course a system with natural rhythmicity is metabolically different from one that contracts only with extrinsic stimulation, so that we should not make too much of these discrepancies. Suffice it to say, however, that we may not as yet conclude directly from increased total metabolism that more thyroxin is required. It is necessary to demonstrate that the increase in heat production in the thyroidectomized animal on exposure to cold is less than that of the normal. Two experiments, neither conclusive, are available on this point.

Hildebrandt in 1921,⁵⁷ using the white rat and a modification of the Haldane technique, compared the basal metabolism for 2 hours in an air chilled chamber at 4 to 6° C with that in a warm room at 19 to 20° C. In 3 animals there was no difference before and 4 to 8 days after thyroidectomy. One animal studied 32 to 45 days after thyroidectomy showed only a slight rise at the low temperature, but not less than some normals. Isenschmid in 1923,⁵⁸ using the Haldane technique, found in 2 rabbits that 5 days after thyroidectomy there was no less response during 2 hours at a temperature of 8 to 9° C than before. In both of these reports the number of animals used is too small, the time after thyroidectomy usually too brief for the animal to be rid of its thyroxin, and the duration of the experiments insufficient to permit the realization of the effects of a substance with so long a latent period as the thyroid hormone.

Thus the physiological evidence for increased thyroid secretion during exposure to cold remains far from satisfactory.* Until better data on this point are forthcoming the interpretation of these experiments must be qualified. The cellular hypertrophy and loss of colloid regularly observed in the thyroid glands of rats exposed to cold represent a preponderant excretion of hormone from the gland; there is no evidence that formation of hormone is impaired, but we must await the demonstration of the greater need for thyroxin by the active tissues before accepting as a strict scientific inference the conception that under these circumstances there is an augmented excretion of the hormone from the gland.

The modifying influence of iodine may similarly be looked upon as meaning an increase of hormone formation relative to excretion and not as necessarily indicating a reduction of the output of the hormone from the gland.

SUMMARY AND CONCLUSIONS

1. Exclusion of light for periods of 10 to 25 days did not produce any striking changes in the thyroid gland of the rat.
2. In confirmation of the work of Cramer, Ludford and Mills, exposure of rats to cold for 10 to 25 days produced hypertrophy,

* Interesting correlative evidence is presented by Dietrich and Schwiegl¹² who demonstrated recently in the anesthetized dog that chilling the animal increases the blood flow to the thyroid.

hyperplasia, loss of colloid and increased vascularity in the thyroid gland.

3. Iodine in doses of 10 y daily diminished or prevented this change.

4. Hypertrophy and loss of colloid in general mean preponderant excretion of the hormone from the thyroid gland, either arising from impaired formation or from excessive excretion of the hormone. The actual amount excreted must be determined on physiological grounds.

5. There are no good reasons for supposing that impaired formation of hormone occurred in these experiments.

6. It is likely that the hypertrophy and colloid loss herein described signify that the thyroid gland was excreting more of its hormone, but the essential demonstration of increased need by the tissues for thyroxin is as yet lacking.

The writer wishes to express appreciation for the stimulation and considerate counsel of Drs. A. J. Carlson, Louis Leiter, O. H. Robertson, Irene Sandiford, Paul Starr and Russell M. Wilder at various points along the way.

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DESCRIPTION OF PLATES

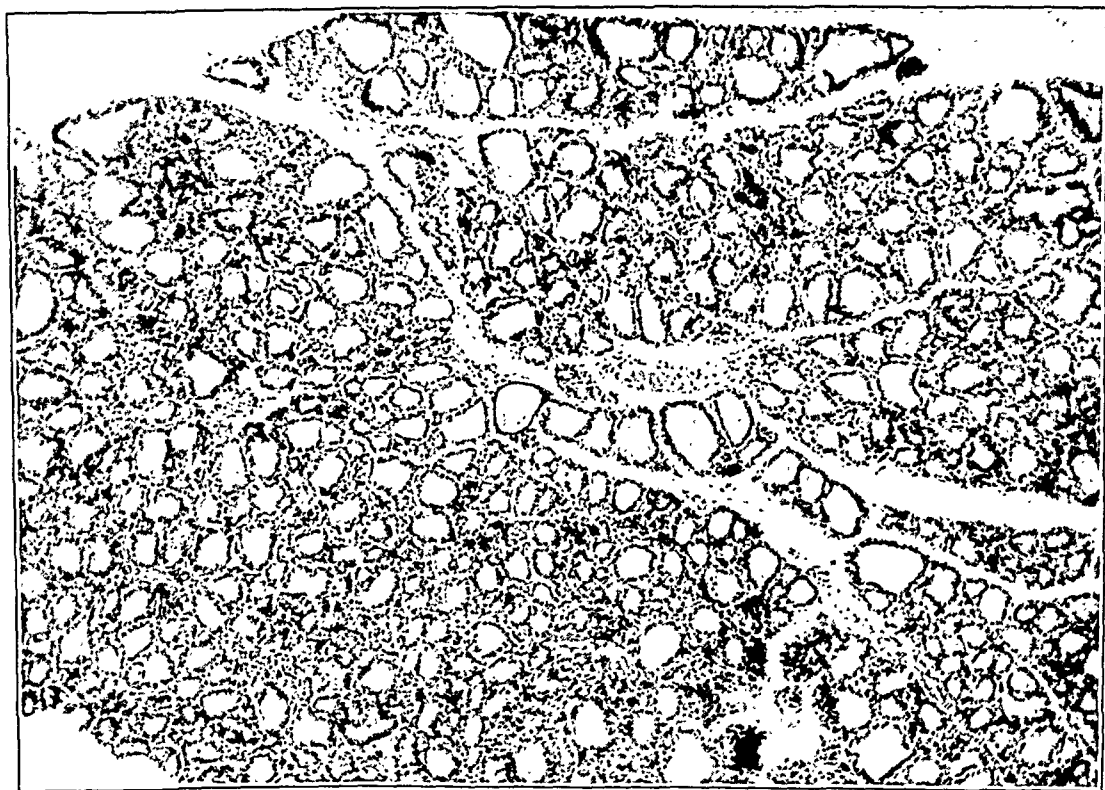
PLATE 59

FIG. 1. Rat 169, kept 25 days at -1°C . Thyroid gland showing hypertrophy of cells and loss of colloid. $\times 70$.

FIG. 2. Rat 177, litter-mate of 169, kept 25 days at 28°C . Normal gland. $\times 70$.



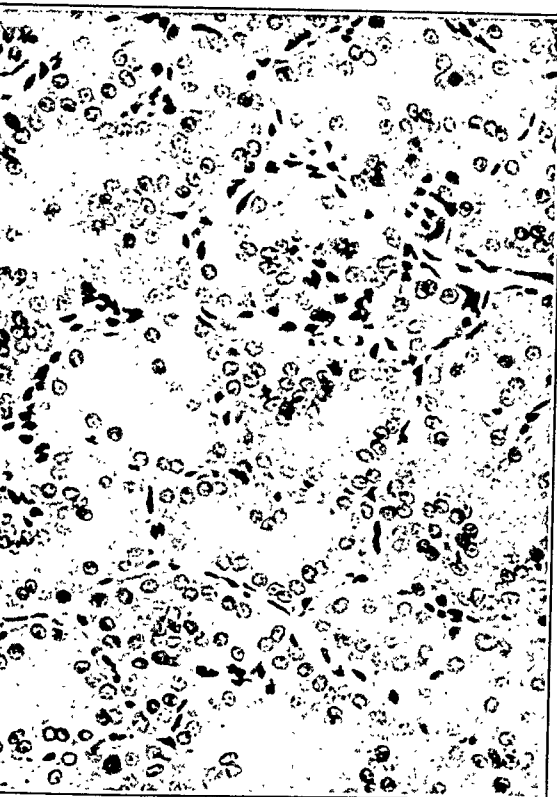
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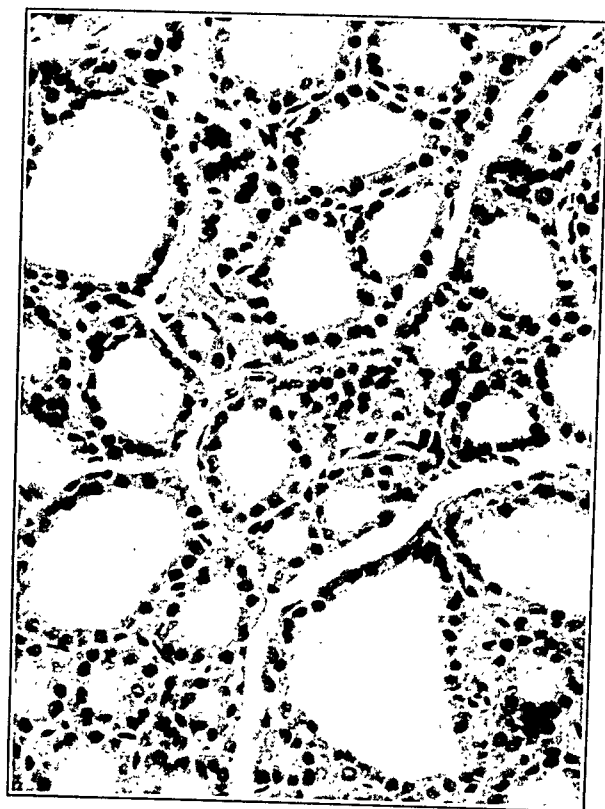
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PLATE 60

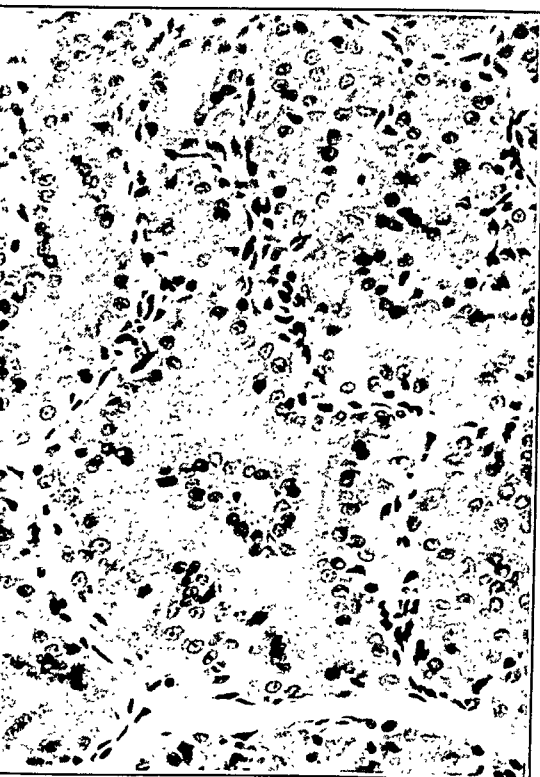
- FIG. 3. Rat 169, kept 25 days at -1° C. Thyroid gland showing hypertrophy of cells and loss of colloid. $\times 275$.
- FIG. 4. Rat 177, litter-mate of 169, kept 25 days at 28° C. Normal gland. $\times 275$.
- FIG. 5. Rat 172, kept 25 days at -1° C. Thyroid gland showing the folding of large follicles with the disappearance of colloid, together with the distention of the vascular channels. $\times 275$.
- FIG. 6. Rat 317, kept 21 days at -4° C, but receiving iodine 10 y daily as potassium iodide. The marked hypertrophy and loss of colloid does not occur. $\times 275$.



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FIBROSIS UTERI *

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This paper presents a study of the collagenous tissue of the myometrium at different age periods and in association with various pathological conditions. An attempt has been made to distinguish the effects of disease from alterations that are merely the result of age. The relation of uterine hemorrhage to fibrosis of the myometrium is discussed.

The relation of fibrosis of the myometrium to uterine bleeding has long been a moot question. Numerous definitions of "fibrosis uteri" have been offered. Shaw (1914) gave a very broad definition: "A uterus enlarged, hard, and containing no tumors, which causes chiefly hemorrhage, pain, leukorrhea, or a combination of these." Bland-Sutton (1904) gave the following description: "A peculiar affection of the uterus, of which the leading clinical feature is uncontrollable menorrhagia, against which both drugs and curettage are of no avail. The disease is chiefly met in women between 35 and 45 years of age. The uterus is large, thick, and tough. Microscopically the muscle tissue is replaced by an abnormal growth of fibrous tissue. The walls of the uterine arteries are thick and the lumina of the vessels narrowed and occasionally obliterated."

The first article on this subject was published by von Scanzoni (1861), who described fibrosis as an anemic, dry, yellow, hard uterus with the arteries and veins reduced in caliber. Finn (1868) and later, Birch-Hirschfeld and Klebs (1896) claimed that the increase in the bulk of the fibrotic uterus was due to muscle tissue, while Klob (1868) insisted it was a direct result of hyperplasia of fibrous tissue. Olshausen (1875) first ascribed uterine hemorrhage to chronic infection of the uterine mucosa, and Brennecke (1882) pointed out the probable relation of the ovary to menorrhagia.

Fritsch (1885) attributed idiopathic uterine bleeding to the pathological increase of fibrous tissue in the uterine wall, which he regarded as the direct result of prolonged inflammatory reaction. Reincke (1897) and Chalmogoroff (1900) stated that uterine bleed-

* Received for publication February 1, 1933.

ing is due to changes in the blood vessels that have lost their contractile and retractile powers, while the uterine wall changes (fibrosis) are coincidental or secondary. Findley (1905), the first American to study this subject, agreed with the above authors.

Solowij (1907), however, insisted that the myometrial changes are first and the arterial changes secondary. Theilhaber and Meier (1902) and Rabinovitz (1910) agree with Solowij and conclude that uterine bleeding is due to a condition of "*insufficiencia uteri*." This is caused by an intermuscular and perivascular development of connective tissue and a corresponding decrease of the muscular elements, decreasing the power of the uterus to contract and control bleeding. Arterial changes are secondary and endometrial changes are unimportant. Theilhaber suggested the possible influence of the ovaries on this condition.

Gardner and Goodall (1906) divided chronic metritis into two types: (a) the simple type in which only the uterus is concerned, and (b) the complicated type, with other organs involved. The bleeding in simple metritis was attributed to two conditions. Corroborating Theilhaber, they believe it is due to a muscular insufficiency of the uterine wall and the arteries. The insufficiency is due chiefly to the restraining influence of the increased amount of non-contractile tissue. This increase of fibrous tissue may be the result of subinvolution, infection, any prolonged congestion or chronic irritation of the uterus such as pregnancy, gestation, adnexal disease, and so on. The second cause of simple metritis is arteriosclerosis.

Rabinovitz (1910) believed that the arterial changes in fibrosis uteri are not arteriosclerosis since the change usually does not begin in the intima. The growth of new connective tissue is seen around the adventitia into which it finally extends. The media undergoes a muscular hypertrophy, probably compensatory, while the intima shows no deviation from the normal. The lumen is held open, while in arteriosclerosis it is closed. Solowij agrees with Rabinovitz. Healy (1915) described two types of arteriosclerosis in women: the one with general arteriosclerosis, involving the heart, liver, kidneys, and so on, leading to a congestion of the pelvic organs from obstruction of the circulation and causing bleeding; the other, with arteriosclerosis of the uterine vessels only. Healy, however, does not discuss the structural alterations in the uterine wall with the above conditions.

Pankow (1909) was the first to doubt the causal relationship between menorrhagia and fibrosis of the myometrium. He proved that myometrial changes may occur without bleeding. Chalfant (1911) after studying eleven cases of "idiopathic" bleeding agrees that microscopically one cannot tell a bleeding from a non-bleeding uterus. Donald (1914) concluded that, aside from the great thickness of nulliparous uteri, there was no difference between the normal and the so-called "fibrosis uteri."

Whitehouse (1914) described a case of fibrosis uteri which he attributed to syphilitic infection. A single instance, however, does not establish a causal relationship between these conditions.

Shaw (1914) believed that the tremendous increase in elastic tissue usually found in multiparous uteri was the most important cause of menorrhagia. He thought that the sheets of elastic tissue about the vessels interfered with their contractility and therefore favored bleeding. He ascribed the bleeding in the nulliparous uterus to endometrial changes.

Geist (1915) after examining 25 specimens with the diagnosis of "fibrosis uteri" decided that he could not find any definite histological lesion to account for the profuse bleeding.

MATERIAL AND TECHNIQUE

For this study 40 uteri obtained at autopsy and 19 uteri removed at operation were examined. This material was divided into the following groups.

The 13 uteri in the first group were from nulliparas ranging in age from 17 to 88 years, dead of accidental causes. In these cases there was no history of local or systemic disease and no lesions were found postmortem, except those due to the fatal accident. There was also no history of abnormal uterine bleeding.

In the second group there are 7 uteri from nulliparas dead of acute infectious processes, *i.e.* pneumonia, diphtheria, liver abscesses, septicemia, and so on. The ages ranged from 23 to 65 years. There was no history of abnormal uterine bleeding in these cases.

In the third group of 14 cases death was caused by a chronic disease, *i.e.* tuberculosis, tertiary syphilis, chronic alcoholism, and so on. The ages in this group ranged from 19 to 78 years. All were nulliparas without a history of abnormal uterine bleeding.

The 10 uteri of the fourth group were operative specimens removed from nulliparas 19 to 45 years of age because of acute pelvic inflammation.

The fifth group includes 15 uteri (9 operative and 6 postmortem specimens) in which there was a history of abnormal bleeding. Six were from nulliparas 21 to 60 years of age, while 9 were from multiparas whose ages ranged from 26 to 53 years and who had had from 2 to 13 pregnancies.

Blocks were taken from various parts of the uterine wall and fixed in both formalin and Zenker's solutions. All sections were stained routinely with Weigert-Van Gieson, azocarmine, Weigert's elastic tissue, and hematoxylin-eosin. For the study of the fibrous tissue in the myometrium and its arteries the azocarmine stain is invaluable, since it demonstrates even the smallest connective tissue fibers. However, it does not differentiate between elastic and collagenous fibers, which is very important in the study of the parous uterus. For this, Weigert's elastic tissue stain or, better still, the combination of Weigert-Van Gieson serves very well. Hematoxylin-eosin, Van Gieson, and the Dominici stains were used to study the cellular structure.

THE MYOMETRIUM OF A NORMAL NULLIPAROUS UTERUS OF A YOUNG WOMAN

As early as 1889 Dittrich criticized the work of the earlier writers because in their studies they did not exclude pathological processes. To avoid this objection the criteria of normal structure have been established by the study of 7 uteri of young women dead of accidental causes, whose histories revealed no recent acute infections, no chronic infections, and no pelvic diseases. The ages varied from 17 to 26 years.

It is with the myometrium that we are principally concerned. In lower mammals this layer can be divided into three distinct strata; however, in the human species the arrangement of muscle fibers is much less regular and the wall cannot be separated into definite subdivisions, although careful examination reveals three indistinct layers intimately blended with one another. The outermost of these is relatively thin, consisting mostly of longitudinal, but also of some circular and oblique bundles. The second is a thick layer of inter-

lacing bundles of more or less circular fibers. Intermingled with these are many large blood vessels supplying both the mucous and muscular coats and giving to this layer the name "stratum vasculare." It merges into the third and innermost layer, stratum submucosum, which also contains many longitudinal fibers.

In the myometrium the smooth muscle fibers are arranged in flattened or cylindrical bundles, separated from each other by bands of connective tissue that extend between the individual muscle cells (Fig. 1). In cross-section the muscle fibers are round, their nuclei are large and well defined, and contain many nucleoli. In longitudinal section it is very difficult to make out the individual muscle fibers with routine stains; but the nuclei appear extremely elongated in the long axis of the fibers, have rounded ends, and contain several nucleoli. There is much variation in the amount of connective tissue between the muscle bundles and muscle fibers. Since in both the outer and inner portions of the myometrium there is a slightly increased amount of connective tissue, the differentiation of the individual muscle fibers is much easier (Fig. 1). In the middle layer, however, the separating tissue is greatly diminished and the muscle cells, which seem to fuse with one another, are practically indistinguishable with the ordinary stains and often even with the Weigert-Van Gieson. However, with the azocarmine the individual muscle fibers, even in the thick middle layer of the myometrium, are well defined by the thin strands of deep blue-staining connective tissue between them.

In the somewhat looser connective tissue between the thicker bundles of smooth muscle, especially in the inner and middle strata of the myometrium, various cells are found. With the Van Gieson and hematoxylin-eosin stain some appear as long flat bodies with several broad, smooth-edged processes, and large oval nuclei with fine chromatin particles and deeply stained nucleoli. These are undoubtedly fibroblasts. There are also some smaller cells, with large dark nuclei and a thin zone of cytoplasm, that resemble lymphocytes. Still other cells of irregular shape are seen that have small kidney-shaped nuclei with a dark coarse chromatin pattern and without nucleoli. These may be called histiocytes. It has been shown by Maximow, Bloom, and Stieve that these three forms of connective tissue cells are not fixed types, but are to some extent interchangeable. During pregnancy there is an increase in the number

of connective tissue cells and then only can the different transition stages from the small (lymphocyte) to the large mononuclear (histiocyte and fibroblast) be seen. Stieve maintains that during gravidity connective tissue cells may become transformed into muscle fibers.

Between the individual smooth muscle cells there is seen in addition to the thin collagenous fibers a dense network of elastic fibers that branch irregularly and pass between the bodies of the muscle cells, forming a fine sheath about each. The amount of elastic tissue in the myometrium diminishes from the periphery toward the mucosa.

AGE CHANGES IN THE NULLIPAROUS UTERUS

It is difficult to obtain uteri in which the alterations are due entirely to age, but 13 cases were available in which death was caused by accident. In these cases the ages varied from 17 to 88 years.

With the advance of age there occurs within the uterine wall a gradual increase in fibrous connective tissue that replaces much of the muscle. The uterine wall of a middle-aged nullipara is quite distinct from that of a younger woman (Figs. 2 and 3). Each individual muscle fiber, even in the muscular middle layer of the myometrium, is definitely enveloped by a heavy sheath of fibrous tissue. The collagenous fibers surrounding the muscle bundles have also increased greatly. The cells, so prominent in the loose connective tissue of a young woman, have disappeared to a great extent and have been replaced by collagenous fibers. The elastic tissue, however, seems unchanged in quantity and distribution. The arteries of the uterus of a middle-aged woman show very little change. The only consistent alteration in the arteries is an increase in the thickness of the adventitia. The muscle fibers of the media are not replaced by collagenous fibers; neither is there any change in the internal elastic lamina. Another minor variation, seen occasionally, is an increase in thickness of the musculo-elastic layer between the elastica interna and the endothelial lining.

In middle life the uterine wall is already beginning to assume the appearance (Fig. 3) so frequently described by many authors as "fibrosis uteri," a change that is found in the myometrium of every older nullipara, regardless of whether disease has been present or not.

The myometrium of an aged nullipara shows still more marked differences (Fig. 4). At this stage the fibrous tissue has increased to such an extent that almost all the muscle fibers have been replaced. It is difficult to differentiate between interfascicular and interfibrillar connective tissue, since they have more or less fused, obliterating the outlines of muscle bundles. The entire uterine wall appears blue with the azocarmine stain. The muscle nuclei are visible after the sarcoplasm has practically disappeared.

The arteries show much less pronounced age changes than the myometrium. The adventitia of the arteries is greatly thickened and blends with the surrounding fibrous tissue. The media and adventitia are no longer separable, because of the replacement of the media by collagenous fibers. Many arteries are seen, however, in which there is no medial fibrosis. With the azocarmine stain these arteries stand out conspicuously as pinkish structures in a solid blue background. Occasionally in the old nullipara there is found medial calcification of the uterine arteries. The intima remains surprisingly free of change in many of the arteries, although the majority show moderate intimal atherosclerosis.

What effect do disease processes have on the age changes in the nulliparous uterus? Seven uteri were examined from nulliparas dead of acute infections: 3 aged 23, 41 and 50 years, dead of pneumonia; 1 aged 30 years, dead of diphtheria; 1 aged 54 years, dead of multiple abscesses of the liver; and 2 aged 42 and 65 years, dead of septicemia. In none of these specimens were there any tissue changes that would enable one to differentiate them from the normal nulliparous uterus. Ten uteri that had been removed because of acute pelvic inflammatory disease were examined and these also were found to be undifferentiable from the normal. It appears, therefore, that acute infectious diseases have absolutely no effect on the uterine tissue, be it a generalized process such as diphtheria or pneumonia, or a localized condition such as an acute pelvic inflammation.

The chronic diseases, however, such as tuberculosis, syphilis and alcoholism do tend to alter the normal physiological age changes. Fourteen uteri of nulliparas dead of various chronic diseases were studied: 3 aged 31, 49 and 78 years, dead of long standing cardiac disease; 5 aged 19, 21, 23, 49 and 50 years, dead of chronic tuberculosis; 4 aged 21, 25, 31 and 55 years, dead of tertiary syphilis; and 2 aged 27 and 50 years, dead of chronic alcoholism. The myometria in

the young nulliparas who had suffered from long-standing chronic diseases did not differ microscopically from those of young healthy virgins. The uterine walls of the older nullipara, however, were a great deal more fibrous than those of the healthy nulliparas of the same age. It would appear that, although chronic diseases have no apparent effect on the uterine wall of a young nullipara, they hasten the normal age fibrosis in the older nullipara.

THE RELATION OF FIBROSIS UTERI TO UTERINE BLEEDING

A discussion of the collagenous tissue changes in the myometrium leads naturally to a consideration of the relation of fibrosis uteri to uterine bleeding. For many years hysterectomies have been performed for uterine bleeding, and in the absence of other changes the bleeding has been attributed to fibrosis of the myometrium. However, several investigators have suggested that fibrosis uteri is chiefly an aging process in the uterine wall and that there is no satisfactory evidence that it is responsible for uterine hemorrhage.

In order to study this problem 15 uteri, in which there was a history of unexplained uterine bleeding, were carefully examined grossly and microscopically. The azocarmine stain proved invaluable in this study because it brings out sharply all the connective tissue fibers in the myometrium.

A study of the uteri from cases of idiopathic bleeding in young women showed no increase of fibrous tissue or abnormal arterial changes that would distinguish them from the normal controls. In the elderly women there was often moderate to extreme fibrosis and a varying degree of arterial change, but these alterations were not more prominent than in the non-bleeders of corresponding age (Figs. 3 and 4). My observations, therefore, support the view that fibrosis uteri is not responsible for uterine bleeding.

Shaw suggested that the increase of elastic tissue around the uterine vessels of the multiparous myometrium interfered with their contractility and was the most important cause of menorrhagia. From the foregoing discussion it is apparent that the predominant change in the nulliparous uterus is an increase of collagenous tissue, but in the parous uterus the outstanding alteration is in the elastic tissue. In microscopic sections of a typical multiparous uterus one readily notes a striking increase of elastic tissue throughout the uterine musculature generally, but more particularly about the

arteries. This tissue is derived primarily from the old uterine arteries during the process of involution, and its amount varies with the number of pregnancies, the health of the individual during her gravid period, and the time of sexual life during which the pregnancies occurred. Szasz-Schwartz (1903), Pankow (1909), and Goodall (1910) have described these elastic tissue changes in the multiparous uterus.

It is evident from microscopic observation that all multiparous uteri have an increase of elastic tissue, but all women who have had children are not afflicted with menorrhagia. In this study it is seen that the myometria of multiparas removed because of uterine bleeding did not contain an excess of elastic tissue over the normal multiparous controls.

The size of the uterus bears some relation to its microscopic structure. The uteri of young nulliparas are of normal size, but a gradual enlargement occurs in middle life as fibrosis of the myometrium develops. However, in the senile period when fibrosis is most marked the uterus decreases in size, finally becoming small and brittle—the typical senile uterus. It is a common belief that the typical example of “fibrosis uteri” is an enlarged organ. This opinion is probably due to the fact that most of the uteri removed because of bleeding are in the physiological stage of enlargement.

SUMMARY AND CONCLUSIONS

1. With the advance of age, there occurs within the uterine wall a gradual increase of collagenous fibers that ultimately replace the greater part of the muscle.
2. Neither localized nor systemic acute processes have any effect on the uterine wall.
3. Chronic diseases have no effect on the uterine wall of young nulliparas, but hasten the age fibrosis in older nulliparas.
4. The uterine arteries show less pronounced age changes than the uterine musculature. The three alterations most commonly found are (1) medial fibrosis, (2) medial calcification, and (3) intimal atherosclerosis.
5. There is no satisfactory evidence that either fibrosis of the uterine wall or increase in elastic tissue, following pregnancy, is responsible for uterine hemorrhage.

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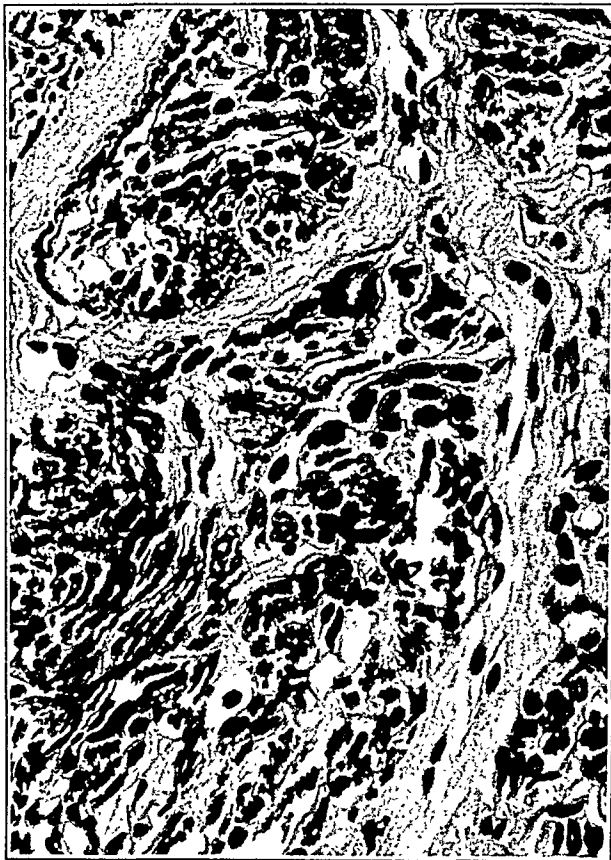
DESCRIPTION OF PLATE

PLATE 61

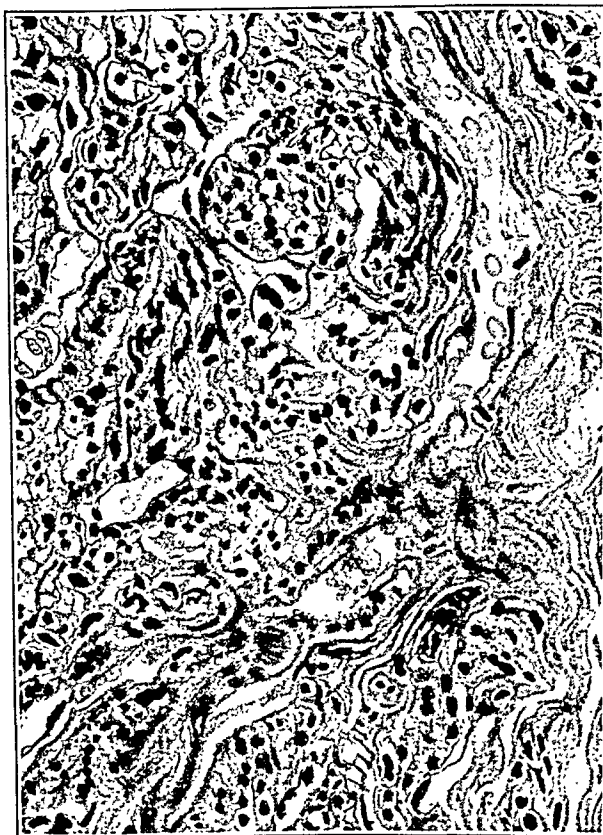
- FIG. 1. Drawing showing a portion of the outer looser layer of the myometrium in a normal healthy nullipara aged 17 years. Azocarmine stain. Note the predominance of the muscle tissue. Fine connective tissue fibers are seen between the muscle bundles. No history of abnormal uterine bleeding. In all four drawings muscle fibers are shown in black, and collagenous fibers in gray. $\times 500$.
- FIG. 2. Drawing showing a portion of the dense middle layer of the myometrium in a normal healthy nullipara aged 30 years. Azocarmine stain. Note the beginning increase of both interfascicular and interfibrillar connective tissue. No history of abnormal uterine bleeding. $\times 500$.
- FIG. 3. Drawing showing a portion of the dense middle layer of the myometrium in a normal healthy nullipara aged 42 years. Note the marked increase of collagenous tissue and its replacement of the muscle. No history of abnormal uterine bleeding. $\times 500$.
- FIG. 4. Drawing showing a portion of the dense middle layer of the myometrium in a normal healthy nullipara aged 72 years. Note the complete fibrosis of the uterine wall. There was no history of uterine bleeding. $\times 500$.



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HEMOPHILUS HEMOLYTICUS ENDOCARDITIS *

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Since the publication in 1923 by Miller and Branch ¹ of a case of bacterial endocarditis caused by the hemolytic hemophilic bacillus, a bacterium that is normally non-pathogenic, no new cases of endocarditis caused by this organism have appeared in the literature. Russell and Fildes ² reported a case of endocarditis caused by the so-called *Bacillus parainfluenzae*, an organism that more closely resembles *Hemophilus hemolyticus* than the ordinary strains of the Pfeifer bacillus. Neither the latter nor the *Bacillus parainfluenzae*, however, is hemolytic.

The hemolytic hemophilic bacillus was first described by Pritchett and Stillman ³ in 1919 in an investigation of the bacteriology of the then pandemic influenza, and was labelled by them "Bacillus X." This microörganism is morphologically and culturally similar to the Pfeifer bacillus, but differs in its ability to hemolyze red blood cells. Stillman and Bourn ⁴ recovered the bacillus from the sputum of patients suffering from acute influenza and lobar pneumonia and from the throat and saliva of healthy persons.

We quote from Miller and Branch a summary of the characteristics distinguishing this microörganism from the influenza bacillus: (a) it causes hemolysis; (b) it occurs only as a saprophyte; (c) it is less strictly dependent on hemoglobin; (d) it has a coarser morphology; and (e) it is slightly more difficult to keep in culture. Bergey ⁵ suggested the name *Hemophilus hemolyticus*, which we shall employ in the present discussion.

We shall proceed to the report of a case of bacterial endocarditis complicated by pregnancy, in which the blood culture yielded *Hemophilus hemolyticus* and also *Streptococcus viridans*. The presence of the former organism was also demonstrated in the microscopic lesions postmortem.

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REPORT OF CASE

Clinical history: The patient, F. F., a married female 32 years of age, an office worker, born in Czechoslovakia, was admitted to the Fourth Medical Division of Bellevue Hospital on November 22, 1931 with a complaint of chills, fever and cough of 3 weeks duration. The patient stated that she had enjoyed good health until the onset of the present illness, which was sudden and occurred about the evening of November 1, when she was taken with a severe chill lasting about 4 hours, followed by fever and sweating. She was prostrated the next morning and called her family physician, who treated her at home for 3 weeks. Her symptoms during that time were chills, fever and cough. The patient had had dyspnea on exertion and occasional edema of the ankles for the past 4 years. There was no previous history of rheumatic fever, chorea, scarlet fever or acute tonsillitis. Her last menstrual period she believed was about 3 months before admission.

Physical Examination: The patient was acutely ill, flushed, dyspneic and slightly orthopneic. Petechiae were present in the right conjunctiva and in the mouth. The teeth were carious and the gums showed marked gingivitis. The heart was not enlarged, the rate was rapid (120) and the rhythm regular. There was a soft blowing systolic murmur at the apex that was transmitted to the axilla. This was heard upon admission and throughout the course of illness. The chest findings consisted of dullness and bronchial breathing over the right lower lobe and a friction rub over the left lower lobe. The spleen was not palpable. The fingers showed slight clubbing and cyanosis. There was slight pretibial edema. On vaginal examination the uterus was felt to be enlarged to the size of a 4 months' pregnancy.

Laboratory Findings: Temperature 103° F, pulse 120, blood pressure on admission 90/60. The urine contained 2 plus albumin and a few red cells per high power field. Hemoglobin 50 per cent, red cells 3,500,000, color index 0.75, leukocytes 12,000, polymorphnuclears 75 per cent, and lymphocytes 25 per cent. Wasserman negative, blood chemistry essentially normal.

Course of Illness: The temperature was of the septic type — 103-4° F in the evenings with morning remissions to about 100° F. Pulse was always rapid, out of proportion to temperature. Blood pressure determinations remained fairly constant, about 120/80. Repeated urine analyses showed 2 plus albumin and casts. On one occasion red blood cells were noticed in the urine.

On November 24 the spleen was enlarged to percussion and on December 1 it became palpable for the first time. New crops of petechiae were discovered almost daily. A friction rub persisted over the left lower lobe. Numerous moist râles were heard from time to time, particularly over the right chest.

On the evening of December 3 the patient had a severe chill and the temperature rose to 105.4° F. New petechiae were noticed in the left conjunctiva. The patient was unable to move her legs and femoral pulsations were absent. A diagnosis was made of a saddle thrombus at the bifurcation of the aorta. Next morning both legs were cyanosed but warm, the body temperature dropped to 95° F and the patient appeared in a terminal toxic state. She died rather suddenly shortly thereafter, the temperature having meanwhile risen to 98.4° F.

The final clinical diagnosis was subacute bacterial endocarditis, saddle thrombus at bifurcation of aorta, and pregnancy.

BACTERIOLOGY

Three blood cultures were taken during life. The first taken on November 24 was negative after 5 days. On November 30 blood was withdrawn and cultured aerobically in dextrose broth, anaerobically in vitamin broth and in plain agar plates. The plates remained sterile for 10 days. After 48 hours spreads from the dextrose broth were stained and examined daily for 10 days. No organisms were obtained in any of the spreads. The anaerobic vitamin broth was examined on the 7th day and smears revealed an occasional small Gram-positive diplococcus. Subcultures were then made on blood-streaked agar plates. Growth appeared on the 4th day as thin, green, non-hemolytic colonies which, when transplanted to vitamin broth, grew in long chains. Blood plates streaked from these broth cultures showed typical colonies of *Streptococcus viridans*.

On December 3 blood was cultured as follows: 5 cc. were placed in each of two vitamin broth flasks and in one dextrose broth flask, and 1 cc. in each of three plain agar plates. The plates yielded no growth after 10 days. Smears of each broth culture were made after 24 hours and showed small Gram-negative coccoid bacilli occurring singly and in pairs. Some of the bacilli stained irregularly, showing slight polar bodies. Cultures in broth at 48-72 hours showed larger, non-mature Gram-negative bacilli.

Subcultures of the broth cultures were made on blood-streaked agar plates, blood-drop dextrose agar, ascitic agar and plain agar plates. No growth was obtained in the two latter. At 48 hours the two former showed several pin-point colonies, each surrounded by a small hemolytic area. After 72 hours the zone of hemolysis became much larger. On blood-drop agar plates the colonies were "influenza-like," minute, round, smooth, transparent and finely granular with a coarse, granular, central heaping. Growth on "chocolate slants" was abundant, gray and moist. No growth of transplants was obtained on plain or ascitic agar slants. Sodium oleate agar containing 1 per cent heated blood showed large, spreading, brown, moist, influenza-like colonies. When they were smeared the organisms appeared larger, were Gram-negative, and occurred in short filaments, pairs and groups.

POSTMORTEM EXAMINATION

Autopsy was performed within 4 hours of the patient's death. The anatomical diagnoses were: pericardial effusion, vegetative endocarditis of mitral valve, focal embolic myocarditis of left ventricle; infarcts of kidney and spleen; thrombosis of both iliac arteries; pregnancy estimated to be about 6 months; a Meckel's diverticulum; petechial hemorrhages in skin, conjunctivae and pericardium.

Autopsy: The body is that of a white female about 35 years of age with a well developed frame in a poor state of nutrition. Reddish purple petechiae are present over the right shoulder, neck and in both conjunctivae and sclerae. The left leg and thigh are deep purple in color. Both legs are slightly edematous. The abdomen is greatly distended. Slight clubbing and cyanosis of digits is observed.

On opening the abdomen the uterus projects from the pelvis to the level of the umbilicus and contains a ballotable fetus.

The chest contains scanty thymic remains, the pleural cavities are dry and the right lung is bound down posteriorly by dense, fibrous adhesions.

Heart: The pericardium contains about 50 cc. of yellow fluid. The heart weighs 375 gm. The wall of the left ventricle appears slightly hypertrophied but the chambers appear normal. Along the line of closure of the aortic leaflet of the mitral valve is a mass of friable vegetations measuring 2 by 1.5 by 1 cm. that are dark red, granular and crumbly in consistence. There are no extensions along the chordae tendineae to the papillary muscles or to the auricular endocardium. The line of closure of the posterior mitral leaflet is thickened and contains some minute vegetations. Some of the chordae tendineae are slightly thickened and shortened. No old or recent aortic, tricuspid or pulmonary valvulitis is present. Coronary orifices are patent. Vessels show no sclerosis. Myocardium is dark reddish brown. Over the anterior surface of the left ventricle near the apex, directly along the course of the anterior descending branch of the coronary artery, is a circumscribed area of pallor and softening without thinning of the wall. There is a similar area over the posterior surface of the left ventricle near the apex along the course of the circumflex branch. These are recent infarctions.

Lungs: The lungs are smooth save for dense fibrous adhesions over the right lower lobe. The bronchi contain a slight amount of

mucopus. Both lungs contain diffusely scattered, subpleural, dark red areas of infarction, presenting a contrast to the pink lung. These are even more noticeable in the parenchyma.

Spleen: The spleen weighs 800 gm. and measures 15 by 9 by 5 cm. Its surface is slate gray with contrasting yellow areas of infarction varying from 1 to 3 cm. in diameter. On section pulp is soft and bright red, with ragged subcapsular infarcts occupying about 50 per cent of the organ.

Liver: The liver is large, yellow and fatty, and weighs 3000 gm.

Kidneys: The kidneys are slightly enlarged. Capsules strip readily exposing a smooth purplish red surface interrupted by depressed, indented, irregular infarcts varying from 1 to 2 cm. in diameter. Cut surfaces are bluish red in color with infarcts cutting deeply into the organs. The kidney substance does not bulge beyond the capsule. The markings are obscured. Pelves appear congested. The glomeruli are not enlarged. No petechiae are present.

Uterus: The uterus contains a well formed fetus 26 cm. in length. Its placenta is intact.

Iliac Arteries: Distal to the bifurcation of the aorta both common iliac arteries are thrombosed as follows: the proximal 2 cm. of the right common iliac is uninvolved; the distal portion contains a firm, yellow-gray thrombus about 2.5 cm. long extending for about 0.5 cm. into the corresponding hypogastric artery. On the left side the clot begins about 0.5 cm. below the bifurcation of the aorta and is about 4 cm. long. The proximal 3 cm. is dark red, the distal cm. yellow and firm, extending for about 0.5 cm. into the left hypogastric artery.

MICROSCOPIC EXAMINATION

Tissues for microscopic sections were removed from the various organs and fixed in 10 per cent formol and in Zenker's fluid and then embedded in paraffin. Heart sections were removed after the routine manner suggested by Gross and coworkers.⁶ These were stained with hematoxylin and eosin, by Giemsa's method, and by Brown and Brenn's picric acid differential method.⁷

Heart: Gram stains of smears from the vegetations reveal large numbers of Gram-negative bacilli arranged in clusters and short

chains. These organisms exhibit bipolar staining and vary considerably in size and length.

The anterior leaflet of the mitral valve is largely replaced by a mass of vegetations in the base of which a remnant of old valve substance can be recognized. This remnant shows no vascularization or evidence of antecedent rheumatic change. The vegetation consists of a crumbly mass of fibrin and leukocytes in the midst of which Giemsa sections disclose the presence of enormous numbers of Gram-negative bacilli. Many are intracellular both in polymorphonuclear leukocytes and in underlying connective tissue macrophages. The deeper zone of the vegetation is composed of cellular granulation tissue.

The posterior mitral leaflet is covered with several minute verrucae that consist of small subendothelial elevations covered with fibrin. The bases of these elevations consist of closely packed fibroblasts. No microorganisms can be found in this leaflet. Between the small verrucae the valve surface is covered with a column of cells forming a palisade usually 2 or 3 rows in thickness. These cells are directed obliquely or at right angles to the valve surface, and are often covered with a thin layer of fibrin. The cells are elongated with large basal nuclei. Within the valve substance appear foci of fibrinoid necrosis. The subendothelial connective tissue is swollen and hyalinized and surrounding these foci are accumulations of round cells. Large numbers of basophilic mast cells lie beneath the endocardium of the left ventricle, which like the valvular endothelium, is often replaced by a cellular palisade. Mast cells also appear throughout the interstitial tissue of the myocardium where, together with polymorphonuclear and mononuclear leukocytes, they surround coronary venules. The vascular endothelium is flattened and beneath it is a row of clearly staining cylindrical cells directed at right angles to the endothelium.

The muscle fibers in the mitral ring are hyalinized. The endothelium of the left auricle appears normal, but the subintimal tissue is infiltrated with fibroblasts, polymorphonuclear cells and histiocytes. No Aschoff bodies are found in the auricular myocardium.

Purulent emboli are seen in the small branches of the left anterior and posterior descending coronary arteries and in small branches to the papillary muscles. These consist of fibrin clumps with necrotic polymorphonuclear cells, but in places contain proliferating fibro-

blasts. The media is destroyed at one side with resultant formation of a mycotic aneurysm. This purulent process undergoes extension into the adjacent myocardium where extensive infarction, muscle degeneration, polymorphonuclear infiltration and scars of granulation tissue are found. Giemsa sections reveal no microorganisms in the infarcted areas.

Lungs: Marantic infarcts occupy large areas of the sections. The smaller bronchi and terminal bronchioles contain plugs of mucopus. The bronchial walls are infiltrated with lymphocytes and plasma cells. Surrounding them are well circumscribed islands of connective tissue densely infiltrated with round cells and containing "glandular structures" lined by cuboidal epithelium.

Liver: The liver sinusoids are packed with every variety of bone marrow cell including all types of granular leukocytes, together with megakaryocytes and large mononuclear cells. The lymphoid elements in the portal spaces are hyperplastic and contain many myeloid cells. There is an astonishing number of immature forms, myelocytes, band forms and metamyelocytes of the neutrophilic and eosinophilic series. These are found in the portal veins where they appear to marginate and invade surrounding sinusoids.

Spleen: The spleen contains wedge-shaped peripheral infarcts. In the splenic arterioles there are granular leukocytes of varying stages of maturity. The lymphoid follicles are hyperplastic. Many leukocytic cells and megakaryocytes of the same type as were present in the liver lie beside the endothelial sinuses of the pulp.

Kidneys: The majority of the glomeruli are intact but in many of them focal embolic lesions are identified by necrosis of one or more tufts. In some instances emboli lie within afferent glomerular arterioles. Some emboli contain bacteria morphologically identical with *Hemophilus hemolyticus*. The capsules of the affected glomeruli are surrounded by zones of polymorphonuclear leukocytes and round cells. A few epithelial crescents are present in Bowman's capsule. There is a moderate degree of tubular degeneration with exudation of albumin into the lumen and occasionally hemorrhage. The vessels appear intact.

Aorta: In sections through each common iliac artery the middle coat is invaded by polymorphonuclear leukocytes separating the smooth muscle fibers. Both the cells and the medial coat are under-

going necrosis. The vasa vasorum contain numerous polymorphonuclear leukocytes in process of margination. Overlying the intima is a huge thrombus which contains no bacteria.

DISCUSSION

So far as can be determined this is the second case of bacterial endocarditis to be reported in which *Hemophilus hemolyticus* has been the cause. There are several features in this case that call for comment and it is proposed to discuss them in the following order: (1) the bacteriology indicating a mixed infection with *Hemophilus hemolyticus* and *Streptococcus viridans*; (2) the association with pregnancy; (3) the probable primary focus; (4) the nature and significance of a myeloid reaction in the liver and spleen; (5) the unusual embolic phenomena; and (6) the relation of the verrucous endocarditis on the posterior mitral leaflet to the vegetative endocarditis on the anterior mitral leaflet.

There can be little doubt that in this case death was due to infection by *Hemophilus hemolyticus*, probably associated with *Streptococcus viridans*. Proof of infection by the former organism consists in the recovery in blood culture, its presence in smears and in the substance of the vegetation, and finally in its presence within the embolic glomerular lesions. Because the valvular and embolic glomerular lesions were partially productive in character and hence of some chronicity, it would seem reasonable to assume that *Hemophilus hemolyticus* was the primary infective agent. Moreover, in terminal invasions associated with endocarditis it is uncommon to find the infective microorganisms deep in the substance of the vegetations, as in this case, although they may occur on the surface.

The rôle of *Streptococcus viridans* is difficult to interpret. Although the organism is the most frequent single causative agent of bacterial endocarditis, its absence from the heart valve in the presence of a bacteremia is unusual. In twelve cases of bacterial endocarditis in which blood culture yielded non-hemolytic streptococci Wright⁸ was able to demonstrate the microorganisms in the valve section in eleven instances. It would seem, therefore, that this organism is to be found in the valve substance in a high percentage of cases where it is the responsible agent and when blood culture is positive. The frequency with which *Streptococcus viridans* is a secondary invader in rheu-

matic and other infections, together with its absence in the histological preparations, are strong arguments in favor of a secondary infection.

Pregnancy as a complication of bacterial endocarditis is of some interest. The literature mentions relatively few cases with this feature. Kobacker⁹ recently reported a case and quoted Walser who, in an extensive review of the literature, was able to find but two bona fide cases of endocarditis lenta complicated by pregnancy. Walser added two cases of his own. Blumer's comprehensive monograph¹⁰ fails to mention the subject, from which it may be deduced that such an association is infrequent. The fetus in our case was well formed and corresponded to that of a 6 or 7 months pregnancy.

The organizing bronchopneumonia suggests itself as a probable portal of entry. The connective tissue reaction in and about the bronchial wall indicated a process of some chronicity. Blumer's analysis mentions the bronchi as a probable focus of infection in five out of fifty-eight cases of bacterial endocarditis.

The myeloid reaction present in the liver and spleen, as revealed by Giemsa's stain, was so striking as to border on the changes of a myelogenous leukemia. *A priori* there are two sources from which bone marrow cells in extramedullary sites may be derived: (1) from preëxisting cells present in those organs by a process of so-called extramedullary myelopoiesis, and (2) as circulating deposits from the bone marrow.

Maximow¹¹ has demonstrated the formation of bone marrow within the kidney of the rabbit after ligation of the renal arteries and vein. He believed that the endothelial cells of the liver, along with the fixed tissue cells or histiocytes, were endowed with unrestricted mesenchymal potentialities, among which was the ability to form hemocytoblasts from which all successive varieties of bone marrow cells could be derived. Whenever extramedullary myelopoiesis occurred myelocytes appeared first, followed in order by megakaryocytes and erythroblasts, but often the erythroblast stage was not reached.

Evans¹² studied the splenic tumors resulting from infections and distinguished a gray and a red variety. The former occurred in bacterial endocarditis and was characterized microscopically by the presence of numerous myeloid forms. These, he believed, arose from mononuclear myeloid cells normally present in the pulp, but indis-

tinguishable by ordinary staining methods. The red variety was typified by the splenomegaly of typhoid fever in which the predominant cell was an endothelial or mononuclear cell.

Histological examination will not absolutely solve the problem of the origin of these extramedullary myeloid cells. We feel, however, that origin from circulatory deposits is the more likely because the myeloid cells could be traced to the splenic arterioles and to the portal veins. The presence of the young myeloid forms indicates a severe and overwhelming demand upon the bone marrow for leukocytic cells which can be met only by discharge of immature forms.

The embolic phenomena here call for some consideration. Embolic deposits in the left coronary artery with accompanying focal purulent myocarditis were present and appeared identical with lesions reported by Miller and Branch, and by Russell and Fildes. These emboli were probably derived directly from the anterior mitral vegetation. Histologically they were identical with the superficial layers of the vegetations. The infrequency of such emboli may be inferred from the failure of Blumer to mention them in his monograph.

The embolic phenomena in the kidneys are the classical focal Löhlein and Baehr¹³ lesions with *Hemophilus hemolyticus* demonstrable in glomerular tufts. The splenic and renal infarcts are typical. The thrombi in the iliac arteries are localized over areas of purulent destruction of the media, probably from small emboli in the vasa vasorum.

Finally it is interesting to contrast the reactions on the anterior and posterior mitral leaflets. The former was essentially an exudative reaction to bacteria, the latter essentially proliferative and bacteria-free. In fact, the gross and the histological appearance of the vegetations on the posterior leaflet was similar to that seen in acute rheumatic valvulitis, a similarity that has previously been recognized by Clawson and Bell.¹⁴ Recently Leary¹⁵ has described a proliferative reaction of the valve surface in rheumatic valvulitis which he has termed a "palisade formation" and which he regards as the earliest valvular response to the action of bacteria. Von Glahn,¹⁶ Swift¹⁷ and others have called attention to focal degeneration of collagen and elastic tissue of the rheumatic valve surrounded by proliferating cells as a pathognomonic feature of rheumatic inflammation and a precursor perhaps to the Aschoff body. Histologically, it will be recalled, the posterior mitral leaflet showed a type of

reaction similar to, if not identical with, the palisade reaction, as well as a fairly extensive area of fibrinoid swelling and degeneration within the substance of the valve surrounded by cellular reaction. The valvular responses of bacterial endocarditis may thus occasionally imitate those of rheumatic endocarditis.

SUMMARY

1. A case of *Hemophilus hemolyticus* endocarditis associated with *Streptococcus viridans* infection is reported. It represents the second recorded instance of infection by the former microörganism.

2. The infection was complicated by pregnancy.

3. A probable portal of entry is demonstrable in the form of an organizing bronchopneumonia.

4. A myeloid reaction is exhibited in the liver and spleen and probably represents the effect of a severe and prolonged over-stimulation of the bone marrow by a bacterial toxin, with a resultant deposition of many immature marrow cells in the hemopoietic organs.

5. Some unusual embolic phenomena are present in the coronary arteries.

6. A villous endocarditis on the anterior mitral leaflet abounding in bacteria is contrasted with a proliferative reaction on the posterior leaflet. This latter reaction, together with a type of recently described "palisade formation," is here shown for the first time in subacute bacterial endocarditis. The existence of an additional lesion, described as a fibrinoid or hyaline swelling and degeneration of collagen valve substance previously demonstrated in rheumatic valvulitis and in rheumatic auricular endocarditis is shown in subacute bacterial endocarditis.

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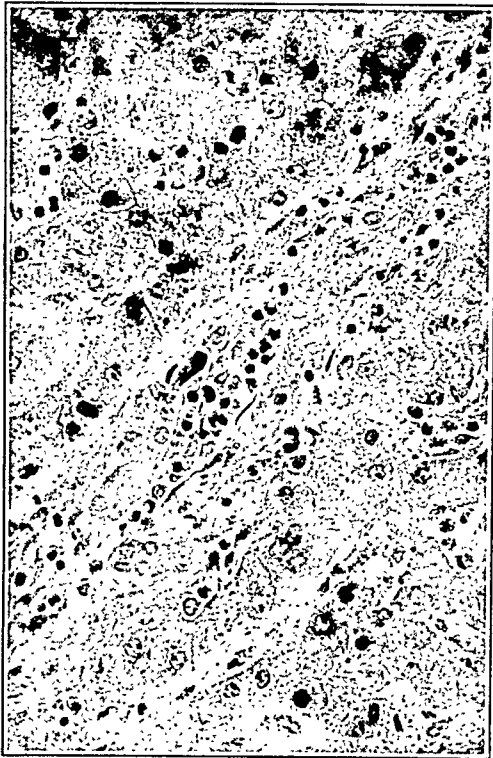
DESCRIPTION OF PLATE

PLATE 62

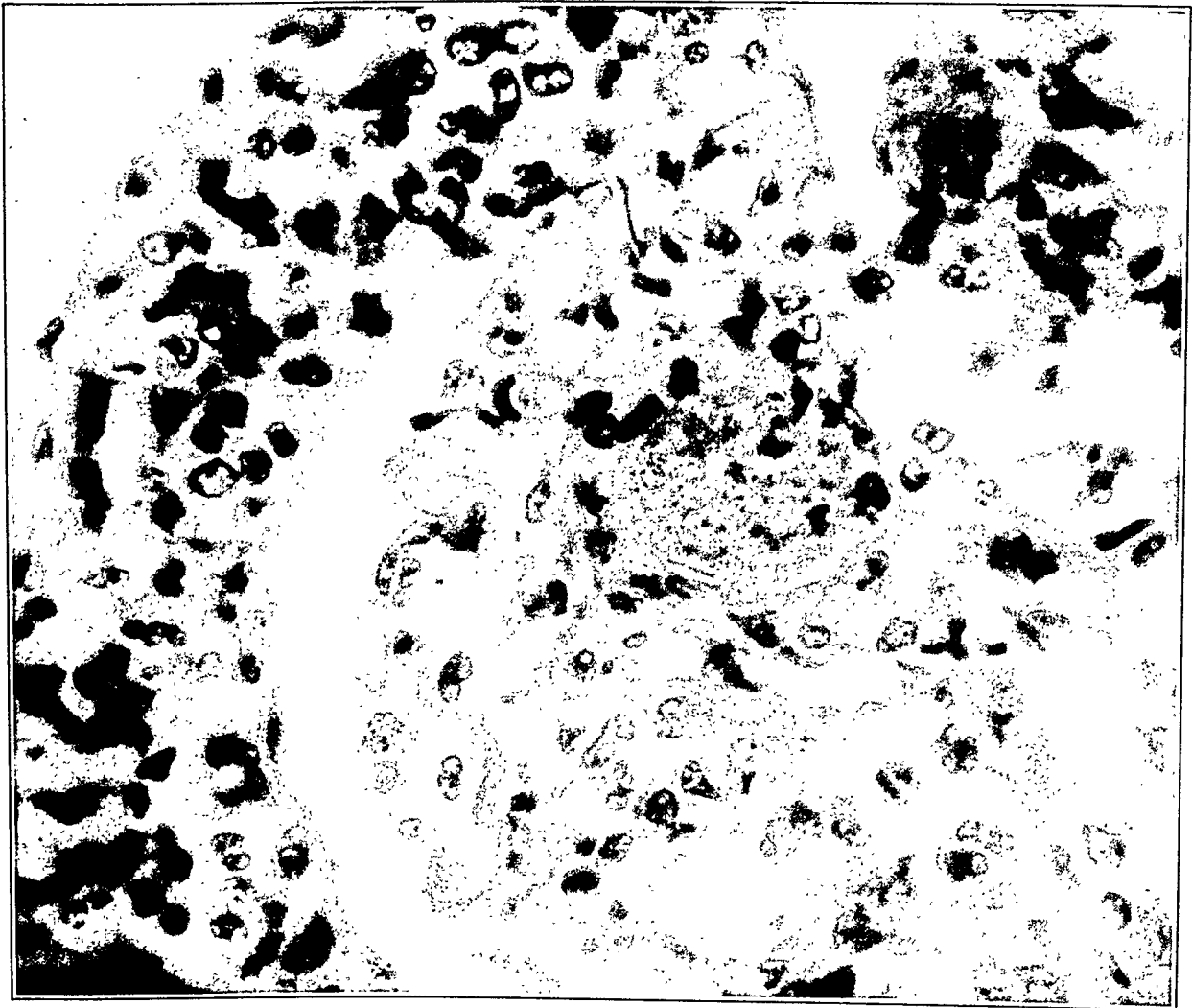
- FIG. 1. A branch of the left anterior coronary artery with an embolic occlusion is seen. The surrounding myocardium is infarcted. Hematoxylin-eosin stain. $\times 70$.
- FIG. 2. Myeloid reaction in liver. Many immature leukocytes may be seen in a portal space and among the sinusoids. Giemsa stain. $\times 150$.
- FIG. 3. Focal embolic glomerulonephritis. Note the bacteria within the afferent arterioles and the surrounding cellular reaction. Hematoxylin-eosin stain. $\times 250$.



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RENAL LESIONS ASSOCIATED WITH MULTIPLE MYELOMA*

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Proteinuria occurs in the great majority of persons afflicted with multiple myeloma, especially in the advanced stages of the disease, and according to the recent literature renal insufficiency is frequently demonstrable toward the end of the illness. In the post-mortem reports that have been published the authors nearly all call attention to some renal lesion, but it would appear that there is no lesion peculiar to multiple myeloma. The purpose of this investigation is to determine the structural alterations in the kidneys responsible for proteinuria and renal insufficiency.

The protein in the urine may consist entirely of the Bence-Jones body, entirely of serum protein, or of a mixture of the two proteins. The more careful investigations indicate that when the Bence-Jones body is present it constitutes the greater part, or all of the urinary protein. The Bence-Jones protein is more frequently found in advanced than in early stages of the disease. In some instances the daily excretion of the protein remains fairly constant over long periods; in other instances it varies in quantity and may be absent for long or short intervals. It is recognized that errors may be made in testing for the Bence-Jones body. It is usually concluded that any protein that does not redissolve on boiling is serum protein, but Hewitt states that Bence-Jones proteins differ in their properties and are not all soluble in boiling solutions. Magnus-Levy states that when a large quantity of protein is found in the urine in a case of myeloma it is very probably Bence-Jones protein chiefly.

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It is difficult to determine accurately the percentage of cases of myeloma in which Bence-Jones proteinuria does not occur at any time. Obviously a negative report is not convincing unless the patient is followed to the end of the illness. Magnus-Levy, in 1932, from a careful survey of the literature concluded that about 73 per cent of the cases show Bence-Jones proteinuria. Geschickter and Copeland gave a somewhat lower estimate — 65 per cent.

The reports summarized in Table I include only cases in which the author gave information concerning renal function, blood pressure, or the histological structure of the kidneys. Reports that give no data on any of these points were not tabulated. The arrangement is in chronological order.

Myeloma without Proteinuria: Four cases have been reported in which no protein was found in the urine (Cases 8, 21, 22, 48). In Geschickter and Copeland's case (Case 48) the patient was living at the time of the report, and it is therefore possible that proteinuria developed subsequently. In the other 3 reports it is not stated how often the urine was examined, or how long before death the examination was made. Microscopic examination of the kidneys was not reported in any of the 3 cases, and no functional studies were made. We do not know, therefore, whether or not there are any functional or structural alterations in the kidneys in cases of myeloma where the urine is normal, but it is probable that no serious lesion is present, since only a slight injury is required to produce albuminuria.

Myeloma with Serum Albuminuria but without Bence-Jones Proteinuria: Another group which may be studied separately is that in which there was serum albumin but no Bence-Jones protein in the urine. Eight cases of this type have been reported (Cases 3, 12, 36, 43, 49, 50, 59, 74) and 2 of our cases fall into this category. In 4 of these only a trace of serum albumin was present, and in another albumin was sometimes absent and sometimes present in small amounts. In 2 instances there was heavy albuminuria. The functional studies on this group are inadequate. In Case 59 the concentration test (specific gravity 1004 to 1012) indicates impaired kidney function. In Perla and Hutner's case (Case 74) the post-mortem report shows clearly a high degree of renal insufficiency. This is the only instance in the literature of proved renal insufficiency in a myeloma without Bence-Jones proteinuria. The authors

interpret the lesion as a nephrotic contracted kidney. We shall discuss this case more fully in a subsequent paragraph.

There are 5 other postmortem reports in this group. One showed pyelonephritis, another many casts with atrophy of the subcapsular zone, and 2 showed only slight changes of no significance. The diagnosis of chronic interstitial nephritis (Case 50) cannot be accurately interpreted. Probably it was an arteriosclerotic kidney.

The rather meager histological studies available indicate that the renal lesions are about the same in the group with only serum albumin in the urine as in those with Bence-Jones proteinuria. Two of our cases belong in this group.

CASE 80: A-30-75. A male, 70 years of age, was under observation the last 2 weeks of his life. The urine on 2 examinations showed casts and a small amount of albumin. Two tests for Bence-Jones protein gave negative results. There was a little residual urine due to hypertrophy of the prostate. The blood pressure was 152/82.

At postmortem the myeloma was found to involve the ribs and vertebrae. The kidneys weighed 180 gm. and 200 gm. each. There were a number of cysts in the cortices, the largest having a diameter of 3 cm., but the greater part of the cortical tissue was intact.

On microscopic examination there are no casts and no atrophic tubules. The glomeruli are normal except for a slight increase of endothelial nuclei.

CASE 81: A-32-691. A male, 59 years of age, was admitted to the hospital May 25, 1931. His symptoms began in May, 1930, and a diagnosis of multiple myeloma was established in October, 1930. Numerous examinations of the urine showed albumin from a faint trace to a small amount. Sometimes no albumin was demonstrable. Repeated careful examinations for Bence-Jones protein gave negative results. The hemoglobin fell from 85 per cent on admission to 69 per cent shortly before death. In June and July, 1930, the icteric index was high, 128 to 160 units, and there was bile in the urine. The blood calcium varied from 10.48 to 15.80 gm. per 100 cc. June 4, 1931, total serum protein 5.85 gm. — albumin 3.61 gm., globulin 2.24 gm. On April 9, 1932, the non-protein nitrogen was 41.6 mg. and the blood pressure was 132/82. There was no edema at any time. Death, April 20, 1932.

At postmortem the weight of the heart was 250 gm. The kidneys weighed 150 gm. and 160 gm. each.

On microscopic examination the large and medium sized arteries show marked intimal thickening. The arterioles are unaffected. There is a definite atrophy of the tubules and glomeruli in the outer

TABLE I

Summary of Reports from the Literature Dealing with Renal Changes Associated with Multiple Myeloma

Case No.	Author	Sex	Age	Proteinuria (no Bence-Jones test)	Serum albumin in urine	Bence-Jones proteinuria	Blood pressure	Phenolsulpho-nephthalein	Blood urea	Non-protein nitrogen	Urea nitrogen	Total serum protein	Serum albumin	Serum globulin	Kidneys
1	Ellinger	M	yrs. 45		?	+		%	mg.	mg.	mg.	gm.	gm.	gm.	A few casts
2	Jochmann and Schumm	F	37		?	+									Amyloid
3	Scheele and Herxheimer	M	50		+	-									Many casts, atrophy of subcapsular zone
4	Collins	M	56	+	?	?									Occasional hyaline glomeruli
5	Tschistowitsch and Kolessnikoff	F	36		?	+									Calcification
6	Hopkins and Savory	F	65		?	+									Slight interstitial fibrosis
7	Austin	M	72		+	+									Atrophy of subcapsular zone
8	Stumm	M	58		-	-									No microscopic
9	Boggs and Guthrie	M	64		-	+	180/?								No postmortem
10	Folin and Denis	M	39		-	+									No postmortem
11	Groat and Brewer	M	37		?	+	135/?	20		81.9	40.8				No postmortem
12	Mieremet	F	57		tr	-									A few casts, small foci of lymphocytes
13	Vance	M	54		?	+									Slight chronic parenchymatous nephritis
14	Froboese	F	52	+											Pyelonephritis
15	Jacobson	M	63		-	+	116/60	0							Each kidney 75 gm., arteriosclerosis
16	Taylor, Miller, and Sweet				+	+									No postmortem
17	Rowe				?	+					6.8	4.8	2.0		No postmortem
18	Glaus	M	67		?	+	145/?								Calcification, amyloid

TABLE I (continued)

Case No.	Author	Sex	Age	Proteinuria (no Bence-Jones test)	Serum albumin in urine	Bence-Jones proteinuria	Blood pressure	Phenolsulpho-naphthalein	Blood urea	Non-protein nitrogen	Urea nitrogen	Total serum protein	Serum albumin	Serum globulin	Kidneys
19	Pepper and Pearce	M	yrs. 52		?	+ + +		%	mg.	mg.	mg.	gm.	gm.	gm.	Arteriosclerosis, casts, degeneration of tubules
20	Beck and McCleary	M	55		+	+									Many casts, chronic diffuse nephritis
21	Wallgren	M	58		-	-									No microscopic
22	"	F	65		-	-	150/?								No microscopic
23	"	M	51		+	+									No postmortem
24	"	M	67		?	+									Slight parenchymatous nephritis
25	"	F	67	+			160/?								No microscopic
26	"	F	50	+			160/?								No microscopic
27	"	F	63	+			105/?								Arteriosclerosis
28	Bloch	F	59		+	+ -									No postmortem
29	Thannhauser and Krauss				+	+	120/68			37.9 202.1		6.12 4.49			Nephrotic contracted kidneys
30	Walters	M	69		-	+	Nm.		Nm.	Nm.					(Living)
31	"	F	42		?	+	124/80 140/80	10 7	74.0	63.0					No postmortem
32	Löhlein	M	50		-	+									Casts, protein crystals, lymphocytic infiltration
33	Oftedal	M	41		?	+	128/96								No microscopic
34	Hansen	M	38		?	+	122/90								No postmortem
35	McConnell	M	49		?	+ + +	190/100								Fibrosis of cortex, atrophy of tubules, casts
36	Wood and Lucké	M	51		Tr.	-									Many hyaline glomeruli
37	Ellermann	M	48	+											Casts, fibrosis, lymphocytic infiltration
38	Stone	M	56		?	+	115/80			37.0					No microscopic
39	Aschner	F	46		+	+ + +	103/?								Embolitic abscess

TABLE I (continued)

Case No.	Author	Sex	Age	Proteinuria (no Bence-Jones test)	Serum albumin in urine	Bence-Jones proteinuria	Blood pressure	Phenolsulpho-nephthalein	Blood urea	Non-protein nitrogen	Urea nitrogen	Total serum protein	Serum albumin	Serum globulin	Kidneys
40	Guggenheimer	M	yrs. 54	+			125/80	%	mg. 28.0	mg.	mg.	gm.	gm.	gm.	Leukemic infiltration, casts, arteriosclerosis
41	"	M	62	+	+	+	140/?		21.5						Arteriosclerosis, lymphocytic infiltration
42	Belden	F	58		+	+					5.0				(Living)
43	Meyerding	M	57		tr	-	150/80								No postmortem
44	Paul and Funk	M	42	+				65 24	48.0						Lymphocytes, casts, dilated tubules
45	Kreuzer	F	56	+			130/65								No microscopic
46	Charlton	F	52		+	+									Interstitial nephritis
47	Kleine	M	53		?	+	145/70								Many casts, severe parenchymatous and interstitial nephritis
48	Geschickter and Copeland	M	44		-	-		Nm.							(Living)
49	"	M	51		+	-	110/85								Pylonephritis
50	"	F	71		+	-	140/90								Chronic interstitial nephritis
51	"	M	66		?	+	104/62	50							Multiple abscesses
52	"	F	37		?	+	130/80								No microscopic
53	"	F	50		?	+									Chronic nephritis
54	"	M	62		?	+	158/108	30							(Living)
55	"	M	59		?	+		9 18	70.0 80.0						(Living)
56	"	M	55	+			135/80	40							Chronic diffuse nephritis
57	Perlzweig and others	M	40		?	+	175/100			34.0		12.32 13.84	1.42 4.06	10.11 9.09	(Living)
58	Marcovici	M	55		?	+	110/80								No postmortem
59	Schittenhelm	F	46		tr	-	180/85								(Living)

TABLE I (continued)

Case No.	Author	Sex	Age	Proteinuria (no Bence-Jones test)	Serum albumin in urine	Bence-Jones proteinuria	Blood pressure	Phenolsulpho-nephthalein	Blood urea	Non-protein nitrogen	Urea nitrogen	Total serum protein	Serum albumin	Serum globulin	Kidneys
60	Bannick and Greene	M	yrs. 47		+	+	110/60 145/100	% 5	mg. 218	mg.	mg.	gm.	gm.	gm.	(Living)
61	"	F	59		?	+	210/110	10 15	104 132	74.0		9.35	2.5	6.5	No postmortem
62	"	M	68		?	+	120/70		100 115						(Living)
63	"	M	49		?	+	145/85	15	84.0						No postmortem
64	"	M	43		?	+	125/45	62 66				6.5 7.3			(Living)
65	"	F	52		?	+	115/70	30	66.0						(Living)
66	"	M	60		?	+	175/115 210/140		62.0						(Living)
67	"	F	69		?	+	185/105	10	46.0						No postmortem
68	"	M	64		?	+	100/65	25		74.0		6.0			(Living)
69	"	M	56		?	+	140/80		50.0						(Living)
70	"	F	70		?	+	130/70	35							(Living)
71	"	M	55		?	+	140/85	45	33.0	40.0		10.08 10.75	4.0 4.45	6.08 6.09	(Living)
72	Hewitt	M	46		-	+		17 34	54.0 77.0			6.31	4.35	1.96	(Living)
73	"	F	66		-	+		55	30.0						(Living)
74	Perla and Hutner	M	71		+	-	135/75		52.2						Casts, marked tubular atrophy
75	"	M	43		?	+	130/80				64.0				Many casts
76	Magnus-Levy				-	+				35.0		6.8			(Living)
77	Mainzer (Ehrich)	M	46		-	+	140/95 165/95		14.8	46.9 89.0		7.38	4.69	2.21	Extensive tubular atrophy due to casts
78	Hallay and Odor	F	38		+	+	140/80			39.0					Arteriosclerosis, subacute glomerulonephritis
79	Buschke	M		+	+		140/70 100/60		62.7 98.0						Many casts, lymphocytic foci

zone of the cortex near the capsule. This alteration is to be attributed to poor blood supply resulting from narrowing of the large arteries. It is frequently found in the senile kidney.

In all the cases in this group, except Perla and Hutner's, the renal lesions are readily explainable as the effects of arteriosclerosis or of complicating infections.

Bence-Jones Proteinuria but No Serum Albumin: In 9 cases the authors state clearly that the Bence-Jones body was the only protein present in the urine (Cases 9, 10, 15, 30, 32, 72, 73, 76, 77). In 3 of these (Cases 15, 72, 77) renal insufficiency was clearly established by functional tests; in 3 others (Cases 30, 73, 76) renal function was good at the time the test was made. Three postmortem reports are available. In Mainzer's case (Case 77) the anatomical basis of renal insufficiency was extensive obstruction of the tubules by casts — the obstruction resulting in extensive tubular atrophy. In Jacobson's case (Case 15) renal insufficiency was apparently due to arteriosclerosis. The kidneys weighed 75 gm. each, the arteries were thick-walled and there were many hyaline glomeruli. No importance was attached to casts. There were no functional studies in Löhlein's case (Case 32), but the tubules were obstructed by crystallized Bence-Jones protein. No doubt a number of other cases belong in this group, since many authors made no attempt to determine whether or not the urinary protein consisted entirely of Bence-Jones body.

In 15 of the tabulated reports, including 3 of our own, both Bence-Jones body and serum albumin were demonstrated in the urine. Usually the amount of serum albumin was very small in comparison with the large amount of Bence-Jones protein, *e. g.* Wright found 0.03 gm. of serum albumin and 0.33 gm. of Bence-Jones protein in 100 cc. of urine. Sometimes serum albumin is absent in most examinations, but present in small amount occasionally, as in 2 of our cases (Cases 82 and 83).

In 36 of the tabulated reports the Bence-Jones protein was demonstrated, but no test was made for serum albumin. Sixteen reports are tabulated, including 6 of our own, which contain some information of value, although the albuminous urine was not tested for the Bence-Jones protein.

Before proceeding to the general discussion the reports of our own cases will be given.

TABLE II

Summary of 11 Cases of Multiple Myeloma Reported in this Paper

Case No.	Autopsy No.	Sex	Age	Proteinuria (no Bence-Jones test)	Serum albumin in urine	Bence-Jones proteinuria	Blood pressure	Phenolsulpho-naphthalein	Non-protein nitrogen	Urea nitrogen	Total serum protein	Serum albumin	Serum globulin	Kidneys
80	30-75	M	yrs. 70		+	-	152/82	%	mg.	mg.	gm.	gm.	gm.	Slight increase of glomerular endothelium
81	32-691	M	59		- +	-	132/82		41.6		5.85	3.61	2.24	Arteriosclerosis
82	26-1096	M	54		- +	+	108/64			45.73				Casts with some tubular atrophy
83	32-930	M	51		- +	+	140/70	63 48	19.2 38.0		6.53	3.39	3.14	Casts with some tubular atrophy
84	31-408	F	38	++			100/60	5		50.0 58.8				Many casts with extensive tubular atrophy (Fig. 4)
85	32-872	M	51		+	++	130/80							Many casts, protein in glomerular capillaries
86	32-1024	M	52	+++					100 200					Protein in glomerular capillaries (Fig. 2)
87	29-1165	M	70	+										Arteriosclerosis, a few casts, protein in glomerular capillaries
88	30-110	M	47			+	115/82							Numerous casts, thickened basement membrane
89	31-311	M	74	+			130/60							Slight increase of glomerular endothelium
90	30-1207	M	74							95.0				Thickened basement membrane

CASE 82: A-26-1096. Male, 54 years of age, admitted to the hospital July 26, 1926. In 1920 a part of the sternum with portions of several ribs was removed because of a tumor of the sternum. This was not examined but was probably myeloma. After this operation he remained well for about 6 years. On admission he was poorly nourished, and complained of pain in the chest and shoulders. Roentgen-ray examination revealed multiple myeloma involving a large number of bones. The blood pressure was 108/64. July 28, blood urea nitrogen was 45.73 mg. Bence-Jones protein was found in the urine on each of 22 examinations. Usually it was the only protein present, but at times there was some serum albumin. There was no edema. Death, Dec. 13, 1926.

At postmortem the myeloma was demonstrated in the skull, ribs, clavicles, innominate bones and femurs. The heart weighed 300 gm. The right kidney weighed 205 gm. and the left 65 gm. The small kidney was covered with deep pits.

Microscopic sections through the pitted areas show wedge-shaped areas of atrophy. The peripheral portions of the tubules near the capsule are collapsed and atrophic, while the deeper portions near the medulla are distended with large casts. Some of the casts are surrounded by giant macrophages. The large atrophic areas that have largely replaced the cortex of the left kidney are clearly due to obstruction of the tubules by casts. The glomeruli in these atrophic areas show a simple atrophy but no hyalinization. In addition to the large atrophic areas there are occasional clusters of hyaline glomeruli with atrophic tubules near the capsular surface. These are apparently due to senile arteriosclerosis. The slight renal insufficiency in this case is therefore attributed to obstruction of the tubules by casts.

CASE 83: A-32-930. Male, 51 years of age, admitted to the hospital Nov. 9, 1931. Illness began in July, 1930, with pain in the right hip. In October, 1930, the pain had become so severe that he could not walk. A spontaneous fracture of the right hip occurred on Jan. 25, 1931. The fracture did not heal and the patient became permanently bedridden. Roentgen-ray examination on admission revealed widespread involvement of the bones with myeloma. Eleven consecutive examinations of the urine during his 7 months stay in the hospital showed the Bence-Jones protein on each occasion. Serum albumin was usually absent, but occasionally present in small amount. On Nov. 9, 1931, the blood pressure was 140/70; phenolsulphonephthalein 63 per cent (2 hours); non-protein nitrogen 19.2 mg.; Rehberg test 103. On May 2, 1932, the non-protein nitrogen was 38 mg.; Rehberg test 31.7; blood calcium 18.2 gm.; total serum proteins 6.53 gm. — albumin 3.39 gm., globulin 3.14 gm.; phenolsulphonephthalein 48 per cent. Death, June 1, 1932.

The Rehberg test is low but the other tests indicate very slight functional disturbance.

At postmortem myeloma was found in the ribs, sternum, innominate bones, femurs, skull and mandible, and there were multiple nodules in the liver with a maximum diameter of 4.5 cm. There was no edema. The heart weighed 380 gm. The kidneys weighed 210 gm. and 250 gm. each. The external surfaces were smooth and the cortices on section were of a light reddish color.

Microscopic examination of the kidneys reveals numerous casts, chiefly in the convoluted tubules. Numerous small areas of tubular atrophy have resulted from obstruction of the tubules by the casts, but the great majority of the tubules are not atrophic. The majority of the glomerular capillaries show a definite irregular thickening of the basement membrane (Fig. 1), such as occurs in lipoid nephrosis and eclampsia (Bell). This alteration in the basement membrane is convincing evidence of injury of the capillary and is an anatomical basis for proteinuria.

CASE 84: A-31-408. Female, 38 years of age, admitted to the hospital March 3, 1931. In July, 1928, she first noticed dyspnea, edema of the legs and weakness. On Aug. 3, 1928, she delivered a full term infant. Edema persisted for several months after delivery and she never regained her former health. A diagnosis of multiple myeloma was established in March, 1929. In June, 1930, there was a spontaneous fracture of the left humerus. Albumin was found in the urine at this time. On admission there was a marked loss of weight, nausea, vomiting and malaise. The blood pressure was 100/60. The urine showed a heavy cloud of albumin. No test was made for Bence-Jones protein. The blood urea nitrogen was 50 mg. On March 6 the blood urea nitrogen was 58.8 mg., and there were clinical signs of intestinal obstruction. The phenolsulphophthalein output was 5 per cent in two hours. Death, March 8, 1931.

At postmortem the intestinal obstruction was found to be due to extensive amyloidosis of the small intestine. The myeloma was widely distributed in the bones. There was no edema. The kidneys were somewhat reduced in size, and on section the cortices were cloudy.

Microscopic examination reveals enormous numbers of casts in the tubules. The portions of the tubules containing casts are greatly dilated but the distal portions near the capsule are collapsed and atrophic (Fig. 4). There are no changes in the glomeruli except an irregular thickening of the basement membrane.

CASE 85: A-32-872. Male, 51 years of age. Onset of illness in October, 1931, with spontaneous fracture of a rib. Admitted to the hospital April 3, 1932, complaining of extreme weakness, pain in the chest and poor appetite. Blood pressure 130/80. The urine contained a large amount of protein that was shown to

be mainly Bence-Jones. Roentgen-ray examination demonstrated myeloma in both humeri, the left scapula, the ribs and the vertebrae. The patient lost weight rapidly and died May 18, 1932.

At postmortem the myeloma was found widely distributed in the bones. There was no edema. The heart weighed 300 gm. The right kidney weighed 225 gm. and the left 300 gm. The external surfaces were smooth and the cortices were pale.

On microscopic examination numerous casts are found in both the convoluted and the collecting tubules. The casts are often partly surrounded by macrophages. In spite of the numerous casts there is very little tubular atrophy. One or two glomeruli in a section show a cast filling the capsular space, *i. e.* between the parietal and visceral epithelial layers. In a few glomeruli the capillaries are partly or completely filled with a precipitated protein such as is shown in Figures 2 and 3. This will be discussed subsequently. There are no convincing changes in the capillary basement membrane. No functional studies were made in this case, but the only alteration tending toward renal insufficiency was tubular obstruction by casts, and these were not present in sufficient numbers to cause a serious functional derangement.

CASE 86: A-32-1024.* The patient, a male 52 years of age, had an acute illness in January, 1931, which was diagnosed influenza by his family physician. He never recovered completely from this illness. His physician prescribed for him occasionally until October, 1931, when he advised extraction of his teeth because of anemia. Eleven teeth were extracted. Bleeding began immediately and continued in spite of all treatment. He had never bled excessively before. He was admitted to the hospital Nov. 9, 1931. The hemoglobin was 35 per cent; erythrocytes 2,000,000; leukocytes 6600 — polymorphonuclears 76 per cent, lymphocytes 20 per cent, no immature leukocytes; platelets 298,000. The bleeding and clotting times were both markedly prolonged. He continued to bleed in spite of numerous transfusions. The blood calcium was normal. On November 11 the non-protein nitrogen was 100 mg.; creatinin, 2.9 mg. On November 17 the non-protein nitrogen was 200 mg.; uric acid 10 mg.; creatinin 5 mg. The urine showed continuously a heavy protein content, as well as numerous hyaline and granular casts. Erythrocytes were rarely seen. There was no test for Bence-Jones protein. Death, Nov. 17, 1931.

The bone marrow of the sternum, ribs, vertebrae, and femurs was hyperplastic and of a red color. Microscopically the growth proved to be a myeloma, but there was relatively little destruction of bone. The heart weighed 450 gm. There was a terminal edema of the lungs

* I am indebted to Dr. A. G. Foord for permission to publish this case.

with bronchopneumonia. The kidneys together weighed 380 gm. The external surfaces were smooth, the cortices on section soft and opaque.

Microscopically there are only a few casts in the collecting tubules. The tubules appear practically normal, but the glomeruli show a very unusual appearance (Fig. 2). The glomerular capillaries are partially or completely filled with a precipitated protein substance. All the capillaries in all the glomeruli are distended, but they contain very few erythrocytes, which is good evidence that they were filled in life with plasma having a high protein content. The fixing reagent (Zenker's fluid) precipitated the protein from solution as it precipitates protein from albuminous urine in the tubules. The protein of the plasma would naturally be more concentrated in the glomerular capillaries where filtration of water is taking place. The partial obstruction of the glomerular capillaries by a highly viscous plasma is offered as an explanation of the renal insufficiency from which the patient died. The substance in the capillaries is not as dense as amyloid. In this kidney an amyloid test was not applied, but in a similar instance (Case 87) the amyloid stain gave a negative result.

CASE 87: A-29-1165. Male, 70 years of age. Complained of pain in the right lumbar and right lower thoracic region. No careful clinical or laboratory studies were made. Albuminuria was continuously present. No test for Bence-Jones body.

At postmortem there was no edema. The heart weighed 375 gm., the kidneys together, 250 gm. The external surfaces of the kidneys were smooth.

On microscopic examination there is found a well defined, narrow, subcapsular zone of hyaline glomeruli and atrophic tubules that is obviously the result of arteriosclerosis. Elsewhere the tubules are normal. There are only a few casts. A few glomeruli show the capillaries distended with precipitated protein (Fig. 3) as described in the previous case. The substance in the capillaries reacted negatively to tests for amyloid.

CASE 88: A-30-1110. Male, 47 years of age, admitted to the hospital Dec. 23, 1929, complaining of weakness, loss of weight, pain in the chest and shoulders, and headache. He had a chronic cough and was dyspneic on exertion. The pain in his chest and shoulders had been present for many months. The blood pressure was 115/82. A tumor mass was recognized in the sternum. The urine

contained a trace of protein and hyaline and granular casts. Death in coma, Jan. 13, 1930. A specimen of urine obtained postmortem showed Bence-Jones protein.

The postmortem revealed multiple myeloma. The heart weighed 355 gm. and was greatly dilated. The kidneys weighed 160 gm. and 165 gm. each. The external surfaces were smooth and the cortices pale.

Microscopic examination reveals numerous small casts in the convoluted tubules, many of which are partially calcified. Some giant macrophages are seen about the casts. The tubules are apparently not completely obstructed by the casts. There are numerous hyaline glomeruli, but the cause of their obliteration cannot be determined with certainty. There is a rather marked intimal thickening of the small arteries which may have caused obliteration of glomeruli. All the other glomeruli are of normal size, but they show a marked irregular thickening of the capillary basement membrane such as is shown in Figure 1.

CASE 89: A-31-311. Male, 74 years of age. His illness began with a severe backache following unusual exertion on Nov. 7, 1930. The pain continued to be intense and sometime in December, 1930, he developed numbness and difficulty in using his legs. On December 11 the urine contained a small amount of protein and the blood pressure was 130/60. No test for Bence-Jones protein was made. Later the lower extremities became paralyzed completely and a large bedsore developed over the sacrum. A diagnosis of "tumor compressing the spinal cord" was made. Death, Feb. 21, 1931.

The postmortem examination revealed a myeloma of the lamina of the eighth thoracic vertebra which had grown into the spinal canal and compressed the spinal cord. No other bones were involved. There was lobar pneumonia of the right lower lobe. The heart weighed 210 gm. The kidneys showed no gross evidences of disease.

On microscopic examination the only alteration is a slight increase of glomerular endothelium which was probably caused by the pneumonia.

CASE 90: A-30-1207. Male, 74 years of age, admitted to the hospital in coma. Death 2 hours later. No history could be obtained. The patient was emaciated and there was incontinence of urine and feces. Examination of the heart and lungs gave no positive findings. The blood urea nitrogen was 95 mg., and the blood sugar 0.082 per cent. No further studies were made.

At postmortem there was no edema. Myeloma of the ribs and sternum was demonstrated. The heart weighed 320 gm., the kidneys 115 gm. each.

Microscopically there are no casts, no atrophic tubules and no calcified structures. The capillary basement membrane in most of the glomeruli shows irregular thickenings such as are shown in Figure 1.

The clinical phenomena that suggest renal disease are albuminuria, hematuria, edema, hypertension and impaired renal function. To what extent are these symptoms and signs found in association with multiple myeloma?

Albuminuria: In rare instances there is no protein of any kind in the urine. Serum albumin may be found alone or with Bence-Jones protein, but it is absent frequently and seldom present in large amount. The urinary protein is chiefly Bence-Jones body in most instances when both proteins are present. The Bence-Jones protein may be excreted by apparently normal kidneys, and its presence does not prove a renal lesion. The presence of serum albumin, however, is satisfactory evidence of injury of the glomerular capillaries, but a very slight damage is sufficient to cause albuminuria and one should not make the diagnosis of nephritis in the clinical sense on the basis of albuminuria alone. In view of the small amount of serum albumin usually found and its frequent absence we should not expect to find a clinical nephritis with myeloma, but merely a mild renal injury.

Hematuria: None of the authors mentions hematuria in a case of myeloma, so that in this respect there is a distinction from glomerulonephritis.

Edema: Edema was present in only 6 out of the 90 cases surveyed. It is found chiefly in the lower extremities and is not prominent. It is probably attributable to malnutrition rather than to renal disease.

Hypertension: The blood pressure has been recorded in 54 of the tabulated reports (46 from the literature and 8 of our own). The highest systolic blood pressures were as follows: 100 to 119 mm. Hg., 12 cases; 120 to 139 mm. Hg., 16 cases; 140 to 149 mm. Hg., 12 cases; 150 to 159 mm. Hg., 4 cases; 160 to 169 mm. Hg., 3 cases; 170 to 179 mm. Hg., 1 case; 180 to 189 mm. Hg., 3 cases; 190 to 199 mm. Hg., 1 case; 200 to 210 mm. Hg., 2 cases. The systolic blood pressure was 150 mm. Hg. or above in fourteen instances — 26 per cent. This is about the incidence of primary hypertension in elderly persons, and therefore elevated blood pressure cannot be attributed to the effects of myeloma unless primary hypertension has been ex-

cluded. There is only 1 postmortem report on a subject with very high blood pressure (Case 35), and the kidneys were not described in detail in this instance. We do not know therefore what the renal lesions were in the cases with high blood pressure. There was a moderate terminal elevation in Mainzer's case (Case 77) in which the characteristic renal lesion of myeloma was pronounced.

Impaired Renal Function: Functional studies were made on 43 of the 90 cases under consideration. The tests are not all satisfactory but they indicate that renal function was normal in 12, slightly decreased in 7, and definitely decreased in 24 cases. It is probable that some of those with normal function would have shown impairment in a later stage of the disease, since renal insufficiency is usually a terminal phenomenon.

It may be concluded that renal insufficiency is common in the advanced stages of multiple myeloma, but in other clinical features there is little resemblance to glomerulonephritis. The absence of edema and the normal or increased serum proteins indicate that the renal lesion is not similar to that of lipid nephrosis. Allard and Weber mentioned a lipemia in their case, but Perlzweig, Delrue and Geschickter found the blood cholesterol 130 mg. There are no other observations on the blood lipoids.

The Structural Changes in the Kidneys: It is commonly stated that a nephritis is found at postmortem in 80 per cent or more of cases of multiple myeloma, but a large variety of minor alterations have been called "parenchymatous" or "interstitial nephritis." The only conclusion justified by a study of the literature is that there is usually some lesion in the kidneys that may or may not be caused by the myeloma.

DISCUSSION

1. We shall first consider the 24 cases in which moderate or severe renal insufficiency was demonstrated by functional tests. In this group 10 postmortem reports are available. In Jacobson's case the lesion was probably arteriosclerosis, since the author described small contracted kidneys with sclerotic arteries and hyaline glomeruli. This was presumably an accidental association of myeloma and arteriosclerosis; there is no evidence that the two diseases are related. Thannhauser and Krauss described the kidneys in a case of

myeloma in which death was due to uremia. The kidneys were small and there was widespread tubular atrophy with normal glomeruli. The authors interpreted the lesion as a nephrotic contracted kidney, and it has been widely accepted as such. However, Ehrlich called attention to Bohnencamp's diagnosis of this case. Bohnencamp examined sections of these kidneys, which he obtained from Aschoff, and interpreted the tubular atrophy as secondary to obstruction of the tubules by casts. The implication is that Aschoff concurred in this opinion. The photomicrographs published by Thannhauser and Krauss show some normal tubules among the atrophic ones, which supports Bohnencamp's interpretation. It is highly probable, therefore, that the tubular atrophy in this widely discussed case was not primary but the result of obstruction by casts.

In Paul and Funk's patient the phenolsulphonephthalein excretion fell to 24 per cent, and the blood urea was 48 mg. There was a heavy cloud of albumin, but no test for the Bence-Jones protein was made. The kidneys were large and pale. There was a diffuse round cell infiltration of the cortex and medulla, and there were dilated tubules with many hyaline and cellular casts. The glomeruli were large and some showed adhesions to the tufts. Extensive deposits of calcium were found in the interstitial tissues of the cortex. The authors made a diagnosis of "subacute nephritis," but the description corresponds to tubular obstruction by casts, with an exudative interstitial nephritis (pyelonephritis).

Perla and Hutner in 1930 reported 2 cases with renal insufficiency:

(a) Male, 43 years of age; blood pressure 130/80; no edema; urea nitrogen 64 mg.; creatinin 5.5 mg.; calcium 14.6 mg.; erythrocytes 1,150,000; hemoglobin 15 per cent. The urine contained large amounts of albumin and of Bence-Jones protein, as well as granular casts. The concentration test gave a specific gravity from 1010 to 1011. At postmortem the kidneys weighed together 220 gm. The cortices on section were of a pale yellowish color. The glomeruli were for the most part intact. The cortical tubules were dilated and filled with casts. In the medulla there was an extensive degeneration of the tubules with replacement by connective tissue. Some tubules were calcified. The authors interpreted the lesion as "chiefly a severe nephrosis," yet the only alteration tending to cause renal insufficiency was obstruction of the tubules by casts.

(b) Male, 71 years of age; blood pressure 135/75; hemoglobin 20 per cent; erythrocytes 1,220,000; blood urea 52.2 mg.; creatinin 5.1 mg.; specific gravity 1012; large quantities of albumin in the urine but no Bence-Jones protein; no casts; no edema. The heart weighed 230 gm. Each kidney weighed 100 gm., the surfaces were smooth and the cortices narrow. Microscopically there was an enormous increase of interstitial tissue in the cortices with marked destruction, atrophy and replacement of tubules. The glomeruli were largely normal. The tubules were strikingly atrophic and some were dilated and filled with hyaline casts. There were clumps of lymphocytes throughout the cortices. The arterioles showed some thickening and were occasionally obliterated. The authors interpreted this case as a marked chronic nephrosis. They considered the atrophy and degeneration of the tubules as primary, yet some of the tubules were obstructed by casts and it seems possible that the authors did not give sufficient consideration to this cause of tubular atrophy.

Buschke in 1932 reported a case of multiple myeloma with some renal insufficiency. Albumin in the urine varied from 0.3 per cent to 1.2 per cent. No test was made for Bence-Jones protein. The last concentration test gave a maximum specific gravity of 1020. The blood pressure ranged from 140/70 to 100/60. Two blood urea determinations gave 98 mg. and 62.7 mg. Hemoglobin 27 per cent; erythrocytes 1,100,000. There was no edema. The kidneys were large. Microscopically all the tubules were filled with albumin and casts. The glomeruli were practically normal. There was arteriosclerosis of the large vessels, but the arterioles were normal. Many of the casts were calcified. There were foci of lymphoid cells in the interstitial tissues. The author regarded the lesion as a slowly progressing nephrotic insufficiency. He was not satisfied with the explanation of tubular obstruction by casts.

Mainzer in 1932 described a case of multiple myeloma with marked renal changes. Male, 46 years of age; blood pressure 140/95 to 165/95; proteinuria, at first all Bence-Jones protein, later some globulin but no serum albumin; blood sugar 1.29 to 2.40 per cent, no sugar in the urine; non-protein nitrogen 46.9 mg. to 89 mg.; blood calcium 11.9 to 12.5 mg.; total serum protein 7.38 gm. — albumin 4.69 gm., globulin 2.21 gm., fibrinogen 0.48 gm. The kidneys weighed 140 gm. and 150 gm. each. Microscopically the cortical tubules were small and separated by connective tissue. Large num-

bers of casts filled the tubules. The author's interpretation was nephrotic contracted kidney.

Ehrich studied the kidneys from Mainzer's case and concluded that the lesion was not nephrosis but obstruction of tubules by casts.

Our case (Case 84) bears a close resemblance to the cases of Thannhauser and Krauss, Perla and Hutner, and Mainzer. The tubular atrophy and renal insufficiency are readily explainable as the result of large numbers of casts. In Case 82 the slight interference with renal function may be similarly explained, but in Case 86 an entirely different process is responsible for renal insufficiency, *i.e.* obstruction of the glomerular capillaries.

It seems highly probable that casts are the chief cause of renal insufficiency resulting from multiple myeloma. Completely obstructed tubules undergo dilatation and atrophy. The atrophy of the tubules is not a primary degeneration but a secondary atrophy from disuse. There is no convincing observation on record of a "nephrotic contracted kidney."

2. In the group in which functional tests showed no renal insufficiency there are no satisfactory postmortem reports in the literature. In our case (Case 81) there was some arteriosclerotic atrophy but no change attributable to myeloma.

3. In 25 of the cases collected in Table I there were no functional studies but the kidneys were examined histologically. Löhlein found the tubules obstructed by crystallized Bence-Jones protein. Scheele and Herxheimer, Beck and McCleary, Ellermann and Kleine, all noted the presence of many casts in the tubules. Jochmann and Schumm, and Glaus, found amyloid disease of the kidneys. Two authors found pyelonephritis. This lesion is particularly apt to develop when there is a paraplegia caused by pressure on the spinal cord, and it may also result from prostatic hypertrophy, since the patients are usually elderly men. Arteriosclerosis is mentioned by several authors as a complicating lesion. The diagnoses of "chronic nephritis," "chronic interstitial nephritis," "chronic diffuse nephritis," and so on, which were made by several authors, cannot be accurately interpreted. Probably these terms were applied to changes mainly of arteriosclerotic nature. Scheele and Herxheimer, and Austin, described sclerosis of the superficial zone of the cortex. This change was also observed in our case (Case 81). It is a typical senile lesion resulting from arteriosclerosis.

In summarizing the structural changes in the kidneys found in association with multiple myelomas, it may be stated that some form of renal lesion has been described in nearly every instance. The alteration is usually of a minor nature, and is most often merely the effect of arteriosclerosis or pyelonephritis. When renal insufficiency is present it is probably caused chiefly by casts that obstruct the tubules and may bring about tubular atrophy in extreme instances. In one of our cases (Case 86) there was obstruction of the glomerular capillaries, apparently by plasma of high protein content.

The prevailing opinion is that the amount of Bence-Jones protein excreted is independent of the protein intake (Hopkins and Savory, Folin and Denis, Groat and Brewer, Walters, and Mainzer). Folin and Denis observed an increase when the patient was on a high protein diet but they considered it a coincidence. Magnus-Levy, however, believes that the Bence-Jones protein is not exclusively an endogenous product, but partly derived from proteins of the food.

It is generally agreed that a small amount of Bence-Jones protein may be found in the blood serum, although some investigators doubt the accuracy of the technical methods that are employed. Jacobson found a heavy protein precipitate when the serum was inactivated at 56° C for thirty minutes. He estimated the Bence-Jones protein in the serum at 7.86 per cent. Perlzweig, Delrue and Geschickter found only a small amount of Bence-Jones protein in the serum and suggested that Jacobson's protein was mainly euglobulin. Other investigators agree that only a small amount of Bence-Jones protein is present in the serum (Taylor, Miller and Sweet, 0.2 per cent; Magnus-Levy, a small amount; Hewitt, a trace; Mainzer, 0.006 per cent).

Twelve observations on the serum proteins are available (see Tables I and II). In 9 instances the total protein was normal or slightly decreased, and in 3 there was hyperproteinemia. In the case reported by Perlzweig, Delrue and Geschickter the total serum protein on three examinations ranged from 12.32 gm. to 13.84 gm.; albumin from 1.42 gm. to 4.06 gm.; globulin from 10.11 gm. to 9.09 gm.; and fibrinogen from 0.79 to 0.69 gm. Their analyses indicated that euglobulin forms a large part of the increased protein in the blood. Bannick and Greene found the total serum proteins in 1 case 9.35 gm. — albumin 2.5 gm., globulin 6.5 gm.; and in another the total protein was 10.75 gm. — albumin 4.45 gm., globulin 6.09 gm. They also found the excess protein to be euglobulin.

The Bence-Jones protein does not accumulate in the blood but appears to be excreted readily by the kidneys. The thick protein solution that filled the capillaries and caused renal insufficiency in our case (Case 86) was probably chiefly globulin and not Bence-Jones protein.

The Bence-Jones protein is excreted through the glomeruli. Allard and Weber injected a dog intravenously with 200 cc. of urine containing a large amount of Bence-Jones body and obtained an abundant "albumosuria." Decastello thought that a normal kidney holds back the Bence-Jones body. After injection into dogs he did not find the protein in the urine unless the kidneys had been previously injured by chloroform. Taylor, Miller and Sweet found that large quantities (5 gm.) of Bence-Jones protein must be injected intravenously into dogs before any of it appears in the urine. When the kidneys were injured with uranium nitrate no Bence-Jones body was excreted. Either the injured kidney was less permeable than the normal, or the foreign protein was broken up and eliminated as simpler bodies. Krauss was able to produce Bence-Jones proteinuria in rabbits by injecting large quantities of the protein intravenously. He expressed the opinion that the injured kidney is more permeable to the Bence-Jones body.

It appears established from animal experiments that the Bence-Jones protein may be excreted by normal animal kidneys when it is injected intravenously in large amounts. When small quantities are introduced they are disposed of in some other way. There is no satisfactory experimental evidence that the injured kidney is more permeable to this protein.

The evidence from clinical sources is very strong that the Bence-Jones protein may pass through normal kidneys. There are many reports in which the urinary protein consisted entirely of the Bence-Jones body, and it may be safely assumed that only a slight injury is necessary to permit the escape of serum albumin. The argument that nephritis is more frequent in myelomas with Bence-Jones proteinuria than in those without it has no weight since the most trivial lesions have been reported as nephritis.

Does the excretion of the Bence-Jones protein injure the kidneys? Krauss believed that this protein is toxic and that it injures the kidneys in its passage through them, but Hewitt, and others before him, have shown that the Bence-Jones protein may be excreted in

large amounts over long periods without the appearance of serum albumin in the urine. This fact seems convincing evidence that the Bence-Jones protein may be excreted over long periods without causing injury to the glomerular capillaries. The renal insufficiency that develops so frequently in multiple myeloma is not due to direct injury of the capillaries or tubules but to obstruction of the tubules by casts. The protein injures the kidneys in this indirect way, not during the process of excretion but afterwards.

How are we to explain the presence of serum albumin in the urine? Serum albumin is sometimes the only protein present and often it is found in small or large amounts along with the Bence-Jones protein. Frequently it is present for many months in the early stages of the disease before the Bence-Jones protein appears. There must be at least a slight injury of the glomerular capillaries to allow the escape of serum albumin, and in several of our cases there are marked alterations of the capillary basement membrane (Fig. 1) that indicate capillary damage. But since serum albumin may be present without Bence-Jones protein in myelomas, and *vice versa*, it may be inferred that the Bence-Jones protein does not produce the injury responsible for serum albuminuria. It is not uncommon to find serum albuminuria in malignant diseases, especially those associated with severe anemia. The capillary injury may be caused by anoxemia or by some toxic substance.

The casts in the renal tubules in myelomas are unusual in that they become permanently lodged and act as foreign bodies. Löhlein called attention to casts that were partly crystallized and to some extent surrounded by giant cells. Since no other protein was present in the urine he concluded that the casts were composed of Bence-Jones protein that was partly in crystalline form. Kleine also noted giant cells in contact with large casts. Ehrich observed transitions between granular protein precipitate and solid casts. He agreed with Löhlein that the casts are composed of Bence-Jones protein since there was no other protein in the urine in his case. Giant cells were noted about the casts.

Casts were found in 7 of our 11 cases, and in 1 they were present in enormous numbers (Case 84). In 3 instances some of the casts were partly surrounded by giant cells. The giant cells are apparently formed by the fusion of macrophages that enter the tubule from without. No casts were found in the 2 cases in which no Bence-

Jones protein was excreted (Cases 80 and 81). In Case 89 there were no casts.

There is evidently a causal relation between multiple myeloma and amyloid disease. Magnus-Levy in 1931 collected 19 cases from the literature in which myeloma and amyloid were associated. The amyloidosis may be generalized (Glaus, Weber), but more often it is found in unusual situations such as intestine, voluntary muscle and bones (Askanazy, Hueter, Freund). In Jochmann and Schumm's case and Glaus' case amyloid was found in the kidneys, but usually the spleen, liver and kidneys are not involved. In our case (Case 84) there were extensive deposits of amyloid in the small intestine that caused intestinal obstruction. Magnus-Levy suggested that the Bence-Jones protein is related chemically to amyloid, but all that has been established is that both substances are proteins of endogenous origin.

SUMMARY

1. Renal insufficiency develops frequently in multiple myeloma, especially in advanced stages of the disease.
2. In some instances renal insufficiency is due to arteriosclerosis, in others it is caused by pyelonephritis resulting from compression of the spinal cord, or from prostatic hypertrophy.
3. The only direct effect of multiple myeloma on the kidneys is due to the formation of tubular casts of Bence-Jones protein that obstruct the tubules and cause tubular atrophy. When large numbers of tubules are obstructed extensive atrophy of the cortex and renal insufficiency ensue.
4. There is no evidence that the Bence-Jones protein injures either tubules or glomeruli. Cortical atrophy is on an obstructive basis and should not be interpreted as "nephrotic contracted kidney."
5. In one instance renal insufficiency was apparently caused by the accumulation of a highly concentrated protein solution in the glomerular capillaries. In two other instances this same appearance was found in a few capillaries.

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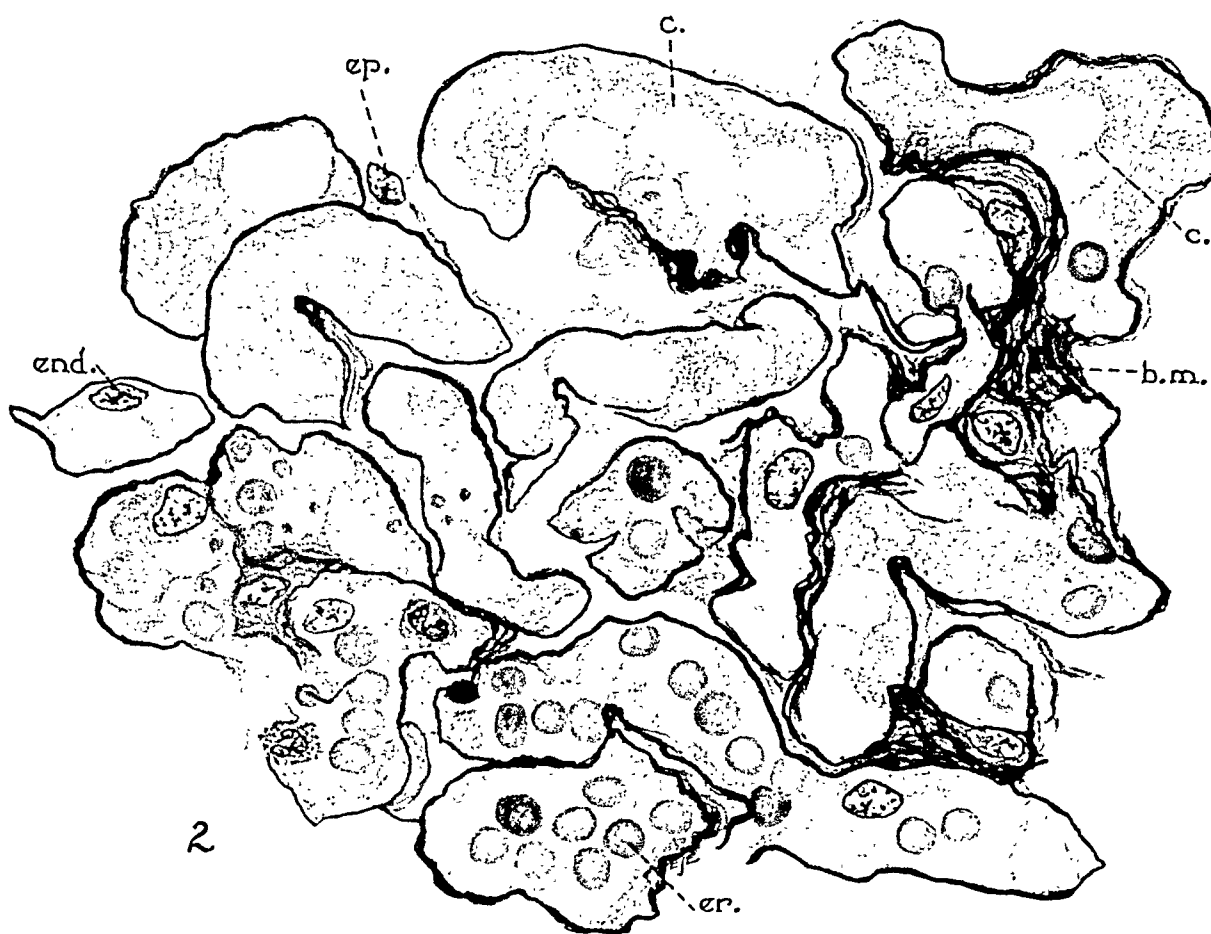
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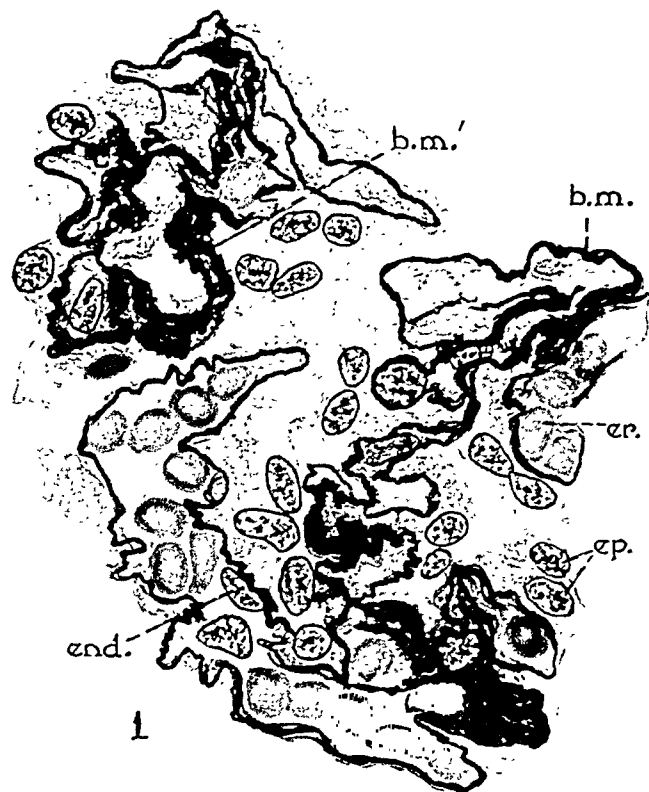
DESCRIPTION OF PLATES

PLATE 63

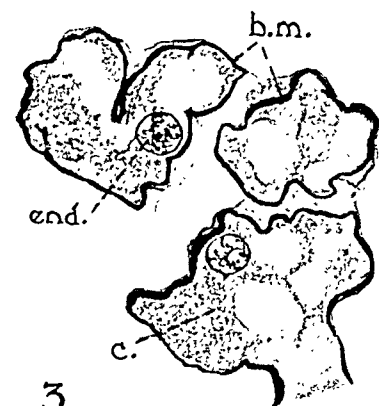
- FIG. 1. A. 32-930. Portion of a glomerulus showing an irregular thickening of the capillary basement membrane: b. m. = normal basement membrane; b. m'. = thickened basement membrane; end. = endothelial nucleus; ep. = epithelial cells; er = erythrocytes.
- FIG. 2. A. 32-1024. Portion of a glomerulus showing all the capillaries distended with protein; c = protein coagulated by the fixative. Other lettering as in Fig. 1.
- FIG. 3. A. 29-1165. Glomerular capillaries filled with coagulated protein; c, as in Fig. 2. Lettering as in Fig. 1.



2



1



3

PLATE 64

FIG. 4. A. 31-408. Area of cortex of kidney showing tubules filled with casts, and atrophy of the tubules proximal to the casts. The glomeruli show only a moderate thickening of the capillary basement membranes.



NEUROBLASTOMA METASTASES IN BONES, WITH A CRITICISM OF EWING'S ENDOTHELIOMA *

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INTRODUCTION

The characteristics of Ewing's sarcoma or diffuse endothelioma of bone, as described by Ewing¹ and other writers, are as follows. The patient is usually a child. The tumour appears most frequently in a short or long bone of a limb and, if in a long bone, affects the shaft diffusely. The onset is with pain and disability, and later enlargement of the segment of the limb. Intermittent mild pyrexia is often present. These features lead in many cases to an initial diagnosis of osteomyelitis, a diagnosis that skiagrams, unless interpreted by workers familiar with this class of tumours, may be held to confirm. Exploratory operation reveals a soft tumour mass surrounding the bone and often infiltrating surrounding soft tissues. Histological examination of an excised specimen reveals a richly cellular tumour composed of closely aggregated, small spheroidal cells of uniform size and shape, each with a spherical hyperchromatic nucleus. These cells appear in diffuse sheets or masses usually devoid of any specific structural arrangement. Purely histological diagnosis, therefore, is not possible. The tumour is highly susceptible to adequate doses of X-radiation, which produces prompt diminution or disappearance of the growth. This favourable response is held to be almost diagnostic of Ewing's tumour. Recurrence, however, is the rule and death usually occurs within two or three years. Almost invariably secondary growths, regarded by some as metastases and by others as multiple new formations, appear in many other bones, especially in the skull and, unlike other bone tumours, Ewing's tumour frequently yields metastases in lymph glands.

The object of this paper is to report a tumour that presented all

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the accepted features of Ewing's tumour, but which careful autopsy study revealed to be one of many skeletal metastases from a small adrenal neuroblastoma.

REPORT OF CASE

Clinical History: J. T., a well developed girl 8 years old, had had previous good health and had suffered no noteworthy illnesses. In June 1932 persistent pain in the right thigh began and in August a swelling of the upper part of the thigh was noticed. The child was admitted to the hospital with the diagnosis "chronic osteomyelitis." Save for a slightly tender, diffuse enlargement of the upper half of the right thigh, general examination disclosed no other abnormalities. The skiagraphic report was: "Area of bone destruction and periosteal reaction in upper third of shaft of femur, appearance suggestive of osteomyelitis. A leukocyte count gave 10,400 cells per cmm. The Wassermann test yielded no reaction. Slight pyrexia was present, the evening temperature frequently reaching 99° F, and sometimes 100° F. On Sept. 17, 1932 the skiagraphic report was as follows: "There has been some extension of the bone involvement, the upper two-thirds of the shaft now being affected." (See Fig. 1.)

On September 21 exploratory operation was performed, revealing a large soft tumour enveloping the shaft of the femur and invading the surrounding soft tissues. A fragment was excised for histological diagnosis. The report on this specimen was: "Richly cellular, round-celled, highly malignant tumour invading skeletal muscle; specific nature cannot be affirmed, possibly a soft tissue sarcoma, possibly a metastatic growth."

A skiagram of the thorax on October 5 disclosed no evidence of lung metastases. On the same day a course of deep X-radiation to the tumour was commenced, and was continued until Nov. 21, 1932. Within four days of the commencement of this treatment pronounced reduction in the size of the growth was apparent and pain and tenderness were much relieved. The rapidity of diminution of the tumour following the initial applications of X-rays was dramatic. During this course of treatment, and thereafter until her death, the patient had increased pyrexia up to 101° F in the evenings, and sometimes to 102° and 103°.

In skiagrams taken on Oct. 27, 1932 no pulmonary metastases were visible but a paravertebral tumour was noted (Fig. 2). The subsequent course was one of emaciation, cachexia and further increase in the size of the femoral tumour. Death occurred on Nov. 29, 1932. The clinical diagnosis was "Ewing's sarcoma of the femur."

POSTMORTEM EXAMINATION

Autopsy was performed six hours after death. There was an enormous, fusiform, soft white growth surrounding the whole of the shaft and neck of the right femur and invading the surrounding muscles. The tumour tissue was easily detached from the femur, the exposed surface of which was eroded and roughened and resembled coarse sandpaper to the touch (Fig. 3). At the lower limit of the

growth there was a slight degree of periosteal new bone formation. The medullary cavity was occupied by tumour tissue. All other bones of the skeleton that were examined presented similar but less conspicuous tumour deposits, situated chiefly beneath the intact periosteum. The inner and outer surfaces of nearly all the cranial bones, mandible, all vertebrae, all ribs, sternum, right humerus, clavicles, pelvis and left femur were involved. Only on the lumbar and thoracic vertebrae did the subperiosteal growths produce tumour masses readily visible externally; elsewhere they formed a thin stratum only discovered on incising the periosteum. The vertebrae and several ribs were sectioned and were found to contain medullary deposits of tumour also. The peripheral bones of the limbs were not examined.

Numerous discrete white tumour nodules were present in the lungs and liver. Both kidneys contained a few tiny points of growth. There were small metastases in the iliac and lumbar lymph glands. The medulla of the left adrenal contained two or three tiny discrete white nodules. The right adrenal was the seat of a group of white nodules occupying the medulla; these, though they appeared to be separate when viewed on a single cut surface (Fig. 4), were connected for the most part with one another, forming a lobulated tumour 3 cm. in maximum diameter with a few smaller, separate satellite nodules. All other organs, including the skull contents, appeared normal.

HISTOLOGICAL EXAMINATION

Microscopic study of haematoxylin-eosin sections of all of the tumours described above, as well as of various viscera, was carried out. Frozen sections of the adrenal growth were stained also by Bielschowsky's method and by Cajal's silver pyridine method for nerve fibres.

The growths in all situations consisted of diffuse masses of rounded cells, each 10 or 12 microns in diameter, with a spherical, deeply stained but vesicular nucleus that sometimes presented a single small nucleolus. Mitotic figures were numerous. The bulk of the tumours exhibited no special arrangement of the cells, but in parts of the right adrenal growth distinct rosette formation was present (Fig. 5). The same feature was observed also, though less clearly, in some of the hepatic metastases. No nerve fibres were found in the Bielschowsky and Cajal-stained sections, and there was no evidence

of any tendency of the tumour cells to differentiate toward the adult nerve cell type.

Of the viscera that appeared macroscopically normal, only the spleen exhibited abnormalities. The vascular spaces of the splenic pulp contained sparsely scattered, small clumps of tumour cells, which were found also in a small accessory spleen.

DISCUSSION

The identity of the tumour in this case is scarcely open to doubt. The primary growth was a highly malignant but small neuroblastoma of the right adrenal that had produced bulky metastases in the other viscera, and especially in the skeleton. The cytology and rosette formation of the adrenal tumour are characteristic of the neuroblastomas of childhood. That the adrenal tumour was small is no argument against its primary character, for the primary growths in the Hutchison² and Pepper³ types of adrenal neuroblastoma are notorious for their small size, compared with that of their metastases in the skull or liver. The possible suggestion that because the left adrenal contained metastatic nodules the right adrenal tumour was also metastatic in nature does not accord well with the much larger size of the right-sided tumour. If, in spite of the cytology of the adrenal growth, it is assumed that the femoral tumour was primary, it is still necessary to admit that all the other skeletal growths were secondary, and this admission greatly detracts from the argument for the primary nature of the femoral tumour. That this tumour was much larger than any other of the skeletal deposits, and that it was the clinically predominant tumour, provide no argument for its primary character, for the Hutchison skull tumours also are in the forefront of the clinical picture and are much larger than the coexistent deposits in other parts of the skeleton.

As regards this clinical predominance of one out of many metastases in bone, a possible cause may be found in the relations of tumour to periosteum in the various situations. It may be that the reason why one metastasis grew luxuriantly while the others remained clinically dormant is that for some cause, possibly traumatic, the periosteum had undergone a solution of continuity at the site of the dominant growth, thereby releasing it from a restraining influence and allowing the neoplasm to flourish unchecked in the

surrounding tissues. In other words, the tumour developed precociously in one particular situation because the restraining periosteum suffered penetration. However, we can see no escape from the conclusion that the case described was one of adrenal neuroblastoma with prolific skeletal metastases, one of which outstripped the others and simulated a large primary tumour of the femur.

Clinically the femoral tumour was diagnosed as a Ewing's sarcoma. How valid was this diagnosis? The accepted characteristics of Ewing's tumour have been summarised in our introduction, and our case presented all of these characteristics. The patient was a child, the tumour affected diffusely a large part of the shaft of a long bone, the onset was with pain and disability, the course of the disease was febrile, the initial clinical and skiagraphic diagnosis was osteomyelitis, metastases developed in other parts of the skeleton including the skull, pulmonary metastases were late in their appearance, biopsy revealed a diffuse spheroidal-celled growth with small spherical hyperchromatic nuclei, and the initial response to X-ray therapy was prompt and striking. In all these respects our tumour conforms to Ewing's description, and further, the skiagraphic features of our case and also the gross appearance of the eroded bone shaft closely resemble those depicted in Ewing's Figures 127 to 129.¹ We believe then that, on all possible clinical grounds, the diagnosis of Ewing's tumour was fully justified, and this diagnosis might easily have received false corroboration from the autopsy, had this not been complete.

It is pertinent then to inquire whether other alleged instances of Ewing's sarcoma or endothelioma may not also have been of a nature similar to ours, and whether adequate autopsy study has been made in any of the cases that have received this clinical designation. In pursuing this inquiry it is disappointing to find that neither in Ewing's accounts nor in those of Connor,⁴ Coley and Coley,⁵ Kolodny,⁶ and Clopton and Womack⁷ is there any detailed autopsy record of any of their cases, and that in the majority of instances the diagnosis rested on clinical and biopsy findings only. Thus, Ewing⁸ records no details of autopsy findings. Connor, discussing 52 bone tumours classified as Ewing's sarcoma in the Registry of Bone Sarcoma, states that "necropsies were done in less than one-third of the cases," and his paper gives no indication as to how complete these autopsies were. Coley and Coley describe many

cases of "endothelial myeloma" diagnosed on clinical and biopsy findings, but autopsy is recorded in only one case (No. 49), of which it is stated: "A necropsy was performed, revealing very extensive metastases in nearly every bone and organ." Kolodny gives a full account of clinical and skiagraphic findings on which, along with the radiotherapeutic response, he would rely entirely for the diagnosis. He considers that clinical distinction from metastatic growths in bones can be made because "metastases occur in the very young or after the age of 40, while most Ewing's sarcomata are seen in early adolescence," a statement with which we venture to think very few pathologists will agree. No autopsies were performed on Clopton and Womack's cases. In the only postmortem record specifically mentioned by Ewing in his *Neoplastic Diseases* (page 361), it is perhaps noteworthy that "retroperitoneal lymphatic metastases were found." On page 352 Ewing says "the diagnosis of endothelioma of bone should not be made until a thorough search for a primary tumor has proved unsuccessful, and this search may, at times, not be regarded as complete without autopsy." We concur with this view, but would modify the latter half of Ewing's statement to read, "this search may *never* be regarded as complete without *thorough* autopsy."

Since no adequate records of autopsies of cases classed as Ewing's tumour are available, we may turn to earlier literature for possible information on the subject. Of much interest is a paper by Roman⁹ who described under the title "myeloplastic sarcoma" two cases of widespread round-celled growths of many bones in children. In one case there was a supposedly metastatic growth involving the left adrenal, and in the other a tumour completely replacing the adrenal. Roman reviewed also several recorded tumours that resembled his own, those of Gussenbauer, Dittrich, and Schmidt, in all of which it is of interest to note that tumour nodules were present in the adrenals. Indeed, it is clear that the tumours described and reviewed by Roman were not, as he supposed, "myeloplastic sarcomas," but instances of adrenal neuroblastoma with widespread bone metastases in the same category as the Hutchison tumours. Evidently Roman was not aware of Hutchison's work of five years previously.² Yet, strange to say, Kaufmann¹⁰ accepts Roman's interpretation.

A purely histological diagnosis of Ewing's tumour is not possible.

All writers on the subject admit that there is nothing distinctive about the cytology or architecture of the growths. As MacGuire and McWhorter¹¹ say: "Most pathologists would call these tumors round-celled sarcomas." However, of possible significance (as regards a neuroblastic nature) is Ewing's¹ observation (see page 359) that "rosette structures without lumina" are present in some of the tumours.

MacGuire and McWhorter confess themselves perplexed by the scope and identity of the group of tumours under discussion, and they conclude: "Ewing's tumor is not yet established as a clinical entity." Hirsch and Ryerson¹² also severely criticise the records of alleged cases, and they suspect that most of them were instances of metastases from undiscovered primary tumours. With this criticism we agree and, while we think it probable that a variety of different conditions has been included in the group of Ewing's tumours, we would urge strongly the claim of adrenal neuroblastoma as the responsible tumour in many instances. This claim is supported by the general characteristics of cytology and metastatic distribution of Ewing's tumours. The multiplicity of skeletal growths, their diffuseness and often predominantly subperiosteal situation, the frequent involvement of the skull, and the frequent presence of lymph gland metastases (a rare feature with all primary bone tumours), are all reminiscent of the malignant neuroblastomas as described by Hutchison,² Tileston and Wolbach,¹³ Frew,¹⁴ Wollstein,¹⁵ and others. It may be recalled that Hutchison's tumours were formerly called "sarcomas" of the skull. We believe that future careful autopsy work will furnish a revelation regarding the Ewing sarcomas similar to that furnished by Hutchison's work for the skull tumours. If, in a case of the Hutchison type, we imagine the bulky skull tumours to be transferred to a long bone, a Ewing tumour would result. The age incidence of the two classes of growth is slightly different, Hutchison's tumours appearing usually in infants, and Ewing's tumours chiefly in older children. It is possible, however, that this very difference may be related in some way to the different sites of preference of the apparent "primary" tumours.

We would draw attention also to the significant fact that "Ewing's sarcoma, primary in the skull" has rarely if ever been recorded, although all writers on the subject comment on the remarkable frequency with which secondary growths appear in the skull. Perhaps

the explanation of this seeming anomaly is that when skull growths are the first to appear the case is recognised correctly as belonging to the Hutchison group and thereby escapes the designation "Ewing's sarcoma."

Finally, we have searched in vain for observations other than our own on the radiosensitivity of neuroblastic tumours, but, from the truly embryonal qualities of their cells and from their high mitotic activity, we would not be surprised to learn that they frequently possess the susceptibility to X-radiation that has been regarded as almost diagnostic of the Ewing tumour.

SUMMARY AND CONCLUSIONS

1. A case is described in which a tumour presenting all the accepted characteristics of a Ewing's sarcoma of bone was shown at autopsy to be one of many metastases from an adrenal neuroblastoma.

2. Review of certain adequately recorded autopsy cases of supposed multiple bone sarcomas leads to the conclusion that these also were instances of adrenal neuroblastoma with skeletal metastases.

3. The term "Ewing's sarcoma," while possessing clinical value as defining a syndrome presented by a certain group of tumours affecting bones, has no established claim as designating a pathological entity.

4. While not denying the *possible* existence of a primary bone tumour presenting the Ewing syndrome, we believe that further study will disclose the metastatic nature of most of the tumours with this syndrome, and we strongly suspect that adrenal neuroblastomas will prove to be the primary growths in many of the cases.

We are indebted to Dr. L. Love for his interest in the skiagraphic aspect of the case reported and for preparing prints of the skiagrams shown in Figures 1 and 2.

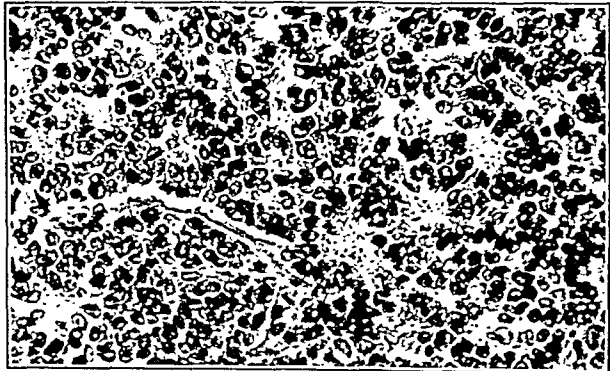
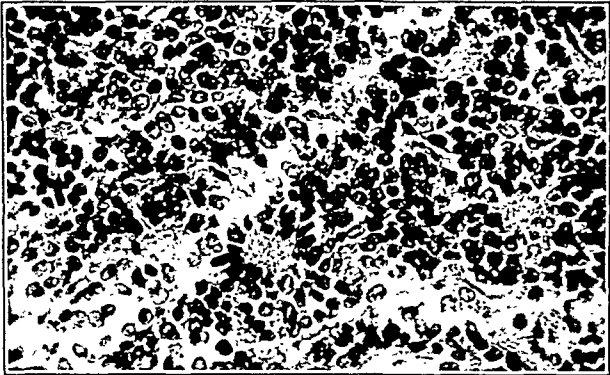
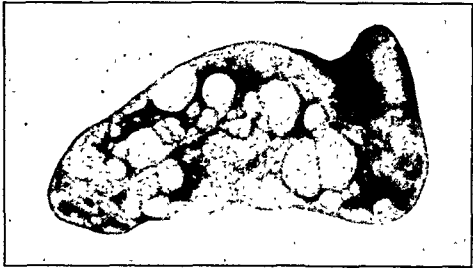
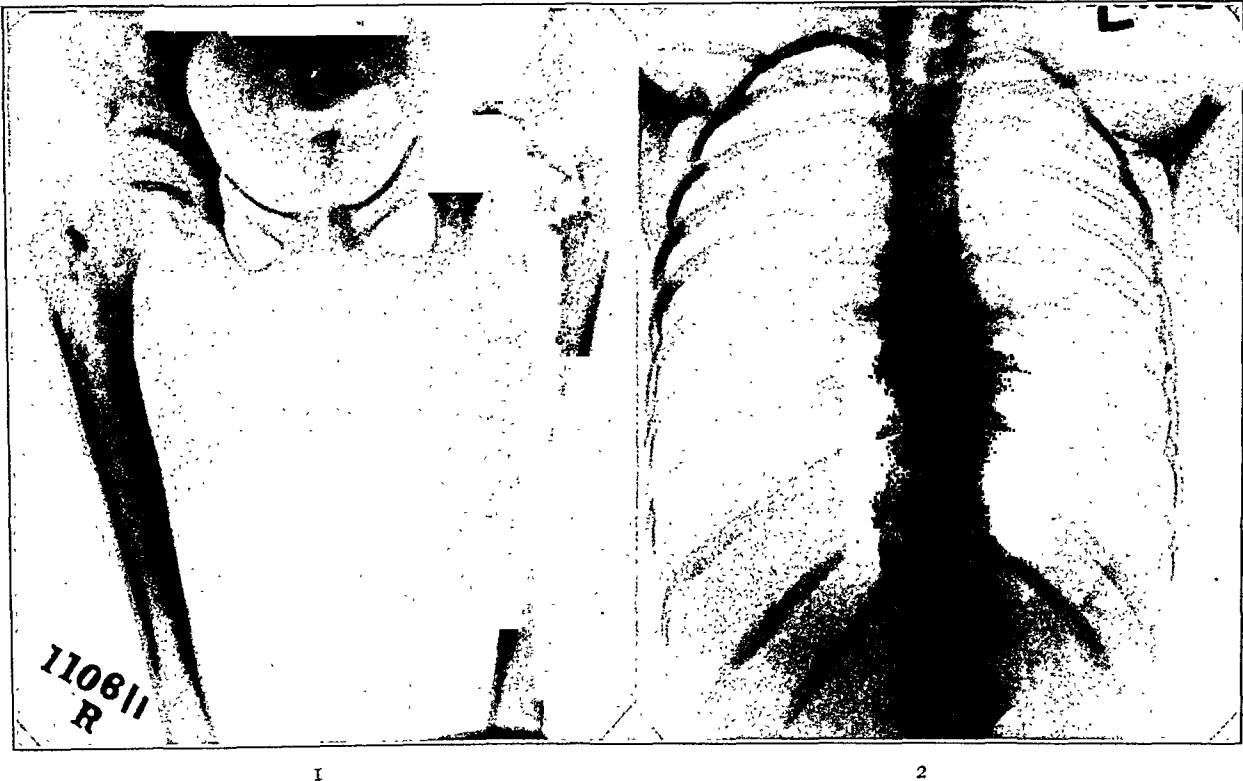
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DESCRIPTION OF PLATE

PLATE 65

- FIG. 1. Skiagram of femur on Sept. 17, 1932.
- FIG. 2. Skiagram of thorax on Oct. 27, 1932, showing paravertebral tumour.
- FIG. 3. Anterior view of femur after removal of soft tumour tissue. Half natural size.
- FIG. 4. Vertical section of right adrenal. Natural size.
- FIG. 5. Two views of haematoxylin-eosin stained sections of adrenal tumour showing rosettes.



EXTREME ALTERATION OF THE AORTIC VALVE IN SYPHILITIC AORTITIS *

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A characteristic gross picture of changes of the aortic valve in some cases of syphilis was described in a previous communication.¹ It was shown that adhesions occur between the lateral portions of the aortic cusps and the corresponding intima of the sinus of Valsalva. These adhesions lead to a spreading of the commissures of the aortic valve and are considered pathognomonic of syphilis. Two cases showing extreme degrees of separation of the commissures, obscurity of a sinus of Valsalva in one instance and transformation of one sinus into a cavity that was filled with blood in the other instance, form the basis of the present communication.

CASE REPORTS

CASE 1. The patient was a 43 year old male. There was clinical evidence of aortic insufficiency over a period of 10 months. The blood Wassermann and Kahn reactions were positive (4 plus).

At autopsy there was edema of the lower extremities, ascites, hydrothorax, and chronic passive hyperemia of the viscera. The aortic changes were characteristic of syphilis. These changes were most pronounced in a segment about 4 cm. in length just above the aortic valve. The mouths of both coronary arteries were markedly narrowed.

The heart weighed 550 gm. The mural endocardium of the inter-ventricular septum in the left ventricle was thickened and showed several endocardial pockets that were open toward the aortic valve. There were occasional yellow plaques of sclerosis on the mitral valve leaflets, but no changes of the pulmonary and tricuspid valves.

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When the aortic valve was opened only two cusps and their corresponding sinus of Valsalva were visible. The posterior cusp on first inspection could not be distinguished and seemed absent, suggesting the possibility of a congenital anomaly. A closer inspection, however, revealed a small, slit-like opening about halfway between the right and left cusps. The distance between the right and left cusps measured 33 mm., while the slit-like opening measured only 6 mm. across and led into a very narrow sinus. The anterior wall of this sinus, therefore, was interpreted as being the remnant of the posterior cusp. The commissure between the left and right cusps was wider than normal, measuring 5 mm. in diameter. The free margins of these cusps were thickened and slightly everted. The commissures were elevated, rough, thickened and partially hyalinized. The aorta just above the commissures revealed many areas of hyalinization, fatty changes, some calcification, and many longitudinal depressed scars and wrinkles with reddish bases. The mouths of both coronary arteries seemed displaced and were much narrower than normal. Both coronary arteries revealed only a moderate degree of arteriosclerosis. The papillary muscles and columnae carnae of both ventricles were hypertrophic and flattened. There were many gray streaks of fibrosis throughout the myocardium.

The area of the aortic ring with the aortic valve was cut into four blocks. All the blocks contained the aortic wall of the sinus of Valsalva and a considerable portion of the adjacent aorta, in addition to the following structures: Block I contained portions of the left and right cusps and the commissure between these cusps. Block II contained the midportion of the right cusp. In Block III a small remaining part of the right cusp, the very large commissure between the right and posterior cusps and also the remnants of the posterior cusp were embedded. Block IV consisted of the commissure between the posterior and left cusps and also of a small portion of the left cusp.

Serial sections of each of the four blocks were made. The sections were stained alternately with hematoxylin-eosin, with a combination of orcein and iron hematoxylin, and according to the Van Gieson method.

HISTOLOGICAL EXAMINATION OF THE AORTIC VALVE

Block I (Portion of Left Cusp, Aorta, Commissure and Portion of Right Cusp): The intima of the aorta revealed a marked thickening with fibrosis and hyalinization. Between the intima and the media many newly formed capillaries were present, many of which were dilated and surrounded by lymphocytes and a few plasma cells. The media showed an interruption of the continuity of the elastic lamellae. Small vessels surrounded by lymphocytes were found in these regions. Serial sections revealed that many of the small vessels crossed the media transversely. The adventitia also showed small sized vessels, the lumina of which were partially or completely obliterated. There was a marked perivascular infiltration of round cells and a few plasma cells, more pronounced than in the other layers, and also a new formation of connective tissue with some hyalinization. Corresponding to the region of the base of the left cusp, small sized blood vessels, many lymphocytes and plasma cells and much connective tissue were found in the intima and media of the aorta extending into the base of the cusp. The intima and media close to the commissure revealed areas characteristic of granulation tissue, namely, many small vessels perpendicular to the surface, that were surrounded by lymphocytes and plasma cells.

Sections that were taken through the lateral portions of the left cusp revealed areas consisting of lymphocytic infiltration and many newly formed capillaries. A few longitudinally cut vessels were found, apparently extending along the lateral portion of the cusp toward the commissure. Close to the commissure much fibrosis and hyalinization were encountered and only relatively few cellular elements. Small blood vessels were still recognizable in these regions. Within the commissure the proliferative changes were much more marked than in other portions of the aorta and in the lateral part of the cusp. The intima showed large areas of hyalinization with calcification and also a new formation of irregularly shaped elastic fibers. Very few small vessels were present in this region, and only occasionally a few lymphocytes could be recognized. The adventitia in these regions showed much fibrosis and hyalinization. Many vasa vasorum, both arteries and veins, were obliterated. Just above the commissure small blood vessels were present, some cut transversely

and some longitudinally, the latter apparently extending from the intima into the adjacent cusp.

Sections taken through the regions of the attached cusp revealed a microscopic opening between the intima and the aortic cusp in areas of the widened commissure in which, grossly, the sinus of Valsalva was not visible. Much hyalinization and fibrosis, but only occasional small blood vessels, were found within the adherent portions of the cusp. Relatively more vessels were found in the distal and proximal portions of the adherent cusp than in its midportion. In only a few sections were a slightly larger number of vessels present at the base of the cusp, these apparently extending from the aorta into the cusp. The lateral portion of the remnants of the right cusp, which was still within the block, revealed at the base loose connective tissue with dilated capillaries and a slight perivascular infiltration.

Block II (Midportion of Right Cusp and Corresponding Aorta): The upper portion of the cusp revealed marked fibrosis and hyalinization, in addition to slight infiltration of lymphocytes and a few endothelial cells. Some of the latter cells were arranged in the form of whorls. The base of the cusp showed dilated capillaries, while the central portion of the cusp revealed a slight increase in connective tissue, but no cellular infiltrations of note. The changes in the aorta were similar to those described before. The region of the orifice of the right coronary artery showed a marked new formation of connective tissue with hyalinization and some calcification. The first portion of the coronary artery very close to its mouth revealed a bulbous-like dilatation.

Block III (Lateral Portion of Right Cusp, Commissure between the Right and Posterior Cusps, Posterior Cusp and Aorta): Some portions of the right cusp revealed capillaries, some of which were surrounded by lymphocytes. Sections that were taken from the commissure itself showed only occasional capillaries extending from the aortic wall into this region and infiltrations of lymphocytes. The central area of the cusp revealed much fibrosis and hyalinization and also a few vessels that extended partly from the proximal and partly from the distal portion of the cusp into the central region. Many of the sections contained a slit-like opening between the cusp and the intima of the sinus of Valsalva, while others showed a new formation of connective tissue with small vessels between the aortic wall of the sinus of Valsalva and the aortic cusp in more localized areas sub-

dividing the space of Valsalva into small compartments. In the sections that were taken from the lateral borders of the cusp a differentiation could not be made microscopically between the thick cusp and the thickened intima of the wall of the sinus of Valsalva. Some of these regions revealed only hyalinization and fibrosis without blood vessels or cellular infiltration. The aortic changes were similar to those described before. The free margin of the posterior cusp revealed slight fibrosis, but few cellular elements.

Block IV (Commissure between the Posterior and Left Cusps, and Portion of Left Cusp and Aorta): Within the commissure blood vessels extended from the intima of the aorta into the adjacent portions of the cusp. Some of them were surrounded by lymphocytes, plasma cells, a few endothelial cells and occasional polymorphonuclear leukocytes. These changes were more marked the closer the sections were taken to the adjacent cusps. A typical granulation tissue was found in this region. Sections that were taken from the base of the cusp also revealed blood vessels and a large number of lymphocytes and endothelial cells. It was demonstrated in the serial sections that some of the blood vessels extended from the aortic wall, at the base of the sinus of Valsalva, into the adjacent basic portion of the cusp. Also, the cellular infiltrations were continuous with those of the aortic wall. The aortic changes were similar to those described before. In several sections, very close to the lateral part of the cusp, the aortic intima was swollen and contained spindle and stellate cells that were separated from one another by an edematous material stained slightly pink with eosin.

CASE 2. This was a colored male, 44 years old.* The clinical diagnosis was syphilitic aortitis with insufficiency of the aortic valve.

At autopsy a typical syphilitic aortitis and a marked narrowing of the mouths of both coronary arteries were found. The commissures of the aortic cusp were much wider than normal. The sinus of Valsalva corresponding to the posterior aortic cusp was almost completely transformed into a cavity by adhesions between the free margin of the cusp and the corresponding intima of the aorta. Only a small midportion of the cusp was free from adhesions. The cavity thus formed was distended because of a small amount of clotted

* This patient was admitted to the Cleveland City Hospital. I am indebted to Prof. H. T. Karsner for permission to publish this case.

blood. Sections were taken from various portions of the aortic valve and adjacent aorta, and stained similarly to those of Case 1.

Histological Findings: The histological examination revealed changes practically identical with those seen in the preceding case. The adventitia and media revealed changes typical of syphilitic aortitis. In the aortic intima there were marked proliferative changes, fibrosis and hyalinization. Small blood vessels were recognized that extended into the commissures. Some of them were surrounded by round cells. Within the widened commissure much old connective tissue was found with slight lymphocytic infiltration. In the region where the cusp comes off the widened commissures the cellular infiltration was more pronounced and a few endothelial and plasma cells were noted. The central portion of the non-adherent cusps revealed no noteworthy changes, while the upper free margins were the seat of simple hyalinization and fibrosis. The base of the cusps showed a few blood vessels, some of which were surrounded by lymphocytes. In the region where only the upper margin of the cusp was adherent to the aorta areas suggesting granulation tissue were found with many dilated blood vessels, lymphocytes, endothelial cells, plasma cells and also a few polymorphonuclear leukocytes.

DISCUSSION

The two cases are similar in many respects. Both revealed a typical syphilitic aortitis grossly and histologically. Both showed a widening of the commissures. In Case 1 the posterior cusp was almost completely attached to the aortic wall of the sinus of Valsalva, leaving only a relatively small midportion free from adhesions. In Case 2 the adhesions were confined to the lateral upper margins of the posterior cusp and did not affect the central portion of the cusp. These changes resulted in a transformation of the sinus of Valsalva into a cavity. The blood clot found within this cavity revealed no evidence of organization. *Intra vitam*, blood must have collected there, which could not be expelled. The blood that filled the cavity evidently prevented the cusp in its entire extent from becoming adherent to the aortic wall, as in the first instance.

The outstanding histological features in both cases are alike. The syphilitic changes in the aorta are the perivascular round cell infiltrations of the adventitia and media, endarteritis obliterans of

the vasa vasorum, and repeated interruption of the continuity of the elastic lamellae of the media.

As compared with the early changes of syphilitic aortitis, which we had occasion to examine in the study referred to previously,¹ the endarteritic changes in general were less frequently encountered in these two cases, whereas the fibrosis of the adventitia was much more marked. This discrepancy of findings in early and in old cases may explain the divergence of opinion as to the primary site of syphilis of the aorta. While Backhaus,² Waite,³ and others believed that obliterative endarteritis of the vasa vasorum is the primary lesion in the aorta, other investigators, and very recently Scherer,⁴ hold the media as the primary site of syphilis. Scherer stated that the primary changes of the media surely are the direct result of the spirochetes, and the damage caused by spirochetes is followed by inflammatory processes. The author, however, failed to demonstrate the spirochetes. Furthermore, his material was taken from patients afflicted with generalized paresis and, therefore, cannot be considered as early syphilis.

The intima, though not revealing lesions morphologically characteristic of syphilis, showed proliferative changes, fibrosis and hyalinization, occasional calcification and granulation tissue. The extension of the granulation tissue into the adjacent and adherent portions of the cusps could be made out clearly. In the central parts and also in the region of the free margins of the cusps, the granulation tissue was absent. In other words, the lateral portions of the aortic cusps were the seat of a chronic inflammation with new formation of vessels. These vessels not only reached the cusps by means of the commissures but also extended from the aorta into the basal portions of the cusps. The extension of blood vessels and groups of small round cells into the base of the aortic cusp was noted by Longcope⁵ as early as 1910. In a recent communication Benedict⁶ classifies two types of inflammatory processes in the aortic valve, namely, an ascending type in which the process spreads through the base of the cusp, and a descending type characterized by the extension of the inflammation through the commissures. It should be emphasized that the evidence presented demonstrates that an inflammatory process arose in the aorta leading to the formation of granulation tissue which, extending to the cusps, formed adhesions between the aorta and the cusps. Also, the newly formed connective tissue that ex-

tended from the aortic wall of the sinus of Valsalva into the cusps subdividing the sinus into smaller compartments in our first case, significant in this respect because a cord of connective tissue in this region can be explained only on a basis of granulation tissue.

Very little can be said about the histogenesis of the valvular lesions from our two cases because the lesions are old. It must be realized that only from early lesions may deductions be drawn as to pathogenesis. In the investigation that was mentioned¹ 71 cases of syphilitic involvement of the aortic valve were studied. In this group some very early cases were included that revealed nutritional disturbances in the intima and lateral portions of the cusps. The resulting degenerative lesions (mucoid degeneration and necrosis) became organized secondarily and were followed by chronic inflammation. Only occasionally could such degenerative lesions be demonstrated in these two cases, apparently because of the long duration of the disease. Krischner⁷ in a recent article considered two possible causative factors — (a) a sensitivity of the tissue of the valve to the syphilitic toxin, or (b) a primary dilatation of the anulus fibrosus with resulting relative hypertrophy of the valve. The separation, wrinklings and thickenings of the commissures, he thought, were due to the stretching of the valvular areas brought about by the relative insufficiency. In both of our cases the dilatation of the aortic ring area was not sufficiently marked to explain the widening of the commissures simply by stretching of this area. Also, the adhesions of the cusps to the aortic wall, in our opinion, cannot be explained as the result of a primary dilatation of the aortic valve region. The findings of typical granulation within the disfigured portions of the aortic valves in both cases speak against a primary mechanical cause. The possible sensitivity of the valvular tissue to a syphilitic toxin cannot be studied with morphological methods. We do not believe such an explanation should be advanced at the present stage of our knowledge of spirochetes and their possible toxins. In our two cases an extension of the granulation tissue was noted not only into those portions of the cusps and commissures that showed the very severe gross lesions, but also into all the commissures. In addition to much fibrosis and hyalinization, found in many sections, blood vessels accompanied by round cell infiltration were noted. These findings also speak against a mechanical genesis of the widening of the commissures and make an inflammatory origin more likely. Whether or

not the hypothesis that suggests the primary changes in the aortic intima and within the aortic cusps are nutritional disturbances followed by chronic inflammatory changes is correct cannot be deduced from the examination of these two old cases. It must be maintained, however, that a careful histological examination of the commissures that are characteristically widened in syphilis reveals inflammatory changes that cannot be explained on a mechanical basis alone.

In regard to adhesions between the aortic cusps and the intima of the aorta it might be of interest to mention Maresch,⁸ who pointed out that during systole the cusp is pressed toward the intima. During diastole, however, the thickened intima above the sinus prevents the regurgitating blood columns from opening the sinus. The cusp therefore remains fixed and later becomes adherent.

Marked deformities of the aortic valve due to syphilis, such as are here described, are very rare. We were able to find only eight similar instances in the literature. Koch⁹ in 1911 reported a case of syphilitic aortic insufficiency in which the left aortic cusp was almost completely adherent to the aortic intima. Only a slit-like opening between the cusp and the sinus of Valsalva was found on section. Paltauf¹⁰ in 1913 reported a similar case in which the right cusp was adherent. In Engel's¹¹ case, also reported in 1913, the sinus of Valsalva was so small as to allow only the introduction of a very thin probe. Maresch in 1930 published four cases, two of which he had observed himself. The other two cases were museum specimens, one dating from the time of Rokitansky. This latter case was, according to Maresch, described as follows: "*Cor quinquagenariae dilatatum ac hypertrophicum cum vasorum truncis. In ostio aortae dilatatae ac incrassatae valvula dextra ex concretionem cum vasis pariete abolita, ostium arteriae coronariae collateralis oblitteratum conspicitur.*" (A dilated and hypertrophic heart with the large vessels of a 50 year old female. At the root of the dilated and thickened aorta the right valve, because of adhesions to the vessel, appears absent and the mouth of the coronary artery obliterated.) The eighth case was reported by Herxheimer¹² in 1931.

SUMMARY

Two cases of syphilitic aortitis with involvement of the aortic valve are reported. In both instances the involvement of the valve had reached extreme degrees, caused a transformation of one sinus of Valsalva into a cavity in one instance, and an almost complete disappearance of a part of one sinus of Valsalva in the other. Only eight similar cases were found in the literature. Histologically granulation tissue and much fibrosis and hyalinization were found within the commissures and the adjacent parts of the adherent aortic cusps. The granulation tissue had spread from the aortic intima through the commissures and also through the base of the cusps into the cusps themselves. The widening of the commissures, which is pathognomonic of syphilitic involvement of the aortic valve, was the result of adhesions between the cusps of the aorta and the corresponding aortic wall. The widening of the commissures, therefore, cannot be explained on a mechanical, but must be explained on an inflammatory basis.

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DESCRIPTION OF PLATE

PLATE 66

FIG. 1. Heart of Case 1. Note the slit-like opening of the sinus of Valsalva, corresponding to the posterior cusp.

FIG. 2. Portion of the aorta and aortic valve of Case 1.



I



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PLATE 67

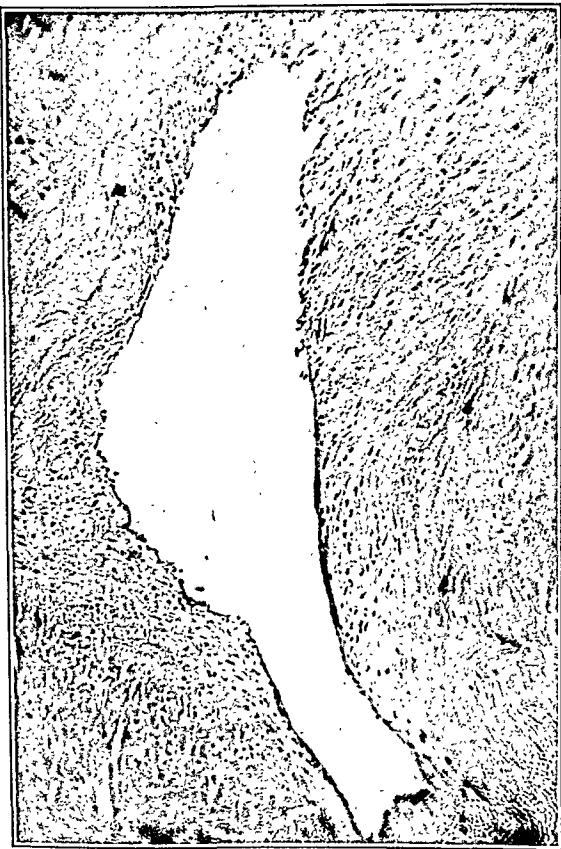
- FIG. 3. Region of the commissure of the aortic valve of Case 1. Note the newly formed blood vessels and the lymphocytic infiltration. Iron hematoxylin-orcein stain. $\times 110$.
- FIG. 4. Base of the aortic valve of Case 1. Note the newly formed blood vessels and lymphocytic cells. Iron hematoxylin-orcein stain. $\times 200$.
- FIG. 5. Slit-like opening of the sinus of Valsalva in Case 1. Hematoxylin-eosin stain. $\times 110$.
- FIG. 6. Aorta and aortic valve of Case 2 cut longitudinally. Note the collection of blood in the sinus space.



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RHEUMATIC HEART DISEASE WITHOUT VALVULITIS*

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The case of rheumatic heart disease to be reported is of unusual interest because of the absence of specific lesions of the heart valves and because an anatomical diagnosis of rheumatic fever depends on the presence of lesions that have only within late years been considered as characteristic of this disease.

In his review Sacks¹ says: "There have been but few reports of cases of proved rheumatic myocarditis in which the valves were entirely free of either recent or old disease. Aschoff's case² of diffuse interstitial myocarditis and Denzer's case³ are examples." Sacks also mentions three cases (Geipel,⁴ Fraenkel,⁵ Libman⁶) in which endocarditis was absent in hearts exhibiting pericarditis accompanied by Aschoff bodies in the myocardium. We have been able to find reports of additional cases in which the valves were said to have been normal, and undoubtedly other cases exist. Following is a brief résumé of these cases.

*Aschoff's Case*² (1904): Death came suddenly three weeks after a hand wound, which meanwhile had healed. Autopsy demonstrated an acute interstitial myocarditis with no sign of endocarditis. Organisms did not grow in cultures and were not demonstrable in sections of heart muscle. The heart muscle showed some necrosis and was infiltrated by many eosinophils and by some adventitial cells, plasma cells and lymphocytes. The most noteworthy thing, however, was the presence of a few characteristic nodules composed of large cells and having an appearance similar to nodules described in cases of rheumatic myocarditis. Such nodules, submiliary in size, lay in close relation to small or medium sized vessels and were composed of extraordinarily large, closely approximated cells having one or more large, slightly indented or irregular shaped nuclei. The centers of the nodules were often formed of poorly staining or apparently necrotic masses of fused cytoplasm.

*Geipel's Case*⁴ (1905): Male, aged 17 years. There was a history of pain in the joints and dyspnea. Autopsy showed fibrinous pericarditis, bilateral fibrinous pleural exudate, edema of the lungs and chronic passive congestion of the viscera. The heart valves were entirely delicate and showed no vegetations. Aschoff bodies were present in the myocardium.

*Geipel's Case*⁷ (1907, 1909): Stone cutter, aged 53 years. There was a history of pain in the joints. After a long walk the patient suddenly fell dead on the street. Autopsy showed great hypertrophy and dilatation of the right ven-

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tricle. Microscopically the myocardium showed Aschoff bodies and a diffuse interstitial myocarditis. The coronary arteries were characteristically involved.

*Fraenkel's Case*⁵ (1912): Female, aged 21 years. History not available. Autopsy showed obliterative pericarditis. Aschoff bodies were found in the myocardium. Streptococci were present in blood culture.

*Poynton's*⁸ (1918): In discussing rheumatic pericarditis Poynton stated that endocarditis is almost an invariable accompaniment, "though occasionally we find no evidence of its occurrence."

*Libman's Case*⁶ (1923): Libman reports: "We have observed one case in which there was a recent pericarditis accompanied by Aschoff bodies in the heart muscle, but not by any lesions of the valves."

*Denser's Case*³ (1924): Female, aged 23 months. Death was due to cardiac failure 5 days after the onset of symptoms. White blood corpuscles 20,000. Temperature did not rise above 100° F. Blood culture sterile. Autopsy (performed by Sacks) showed an enlarged heart with a few hemorrhagic areas on the visceral pericardium. The tricuspid, pulmonary and aortic valves were normal. The mitral valve was not narrowed, its edges were somewhat thickened but there was no evidence of either past or present endocarditis. The left ventricle was hypertrophied and the muscle was flabby and showed tigering. Microscopic examination of the heart muscle showed Aschoff bodies. The heart muscle was edematous and occasional areas of degeneration with cellular infiltration were found.

*Swift's Cases*⁹ (1924): Swift reports: "Recently, two fatal cases have been brought to our attention in which myocardial weakness was the sole clinical picture, and postmortem the only distinct lesions were Aschoff bodies widely disseminated throughout the heart muscle."

VonGlahn and Pappenheimer: VonGlahn¹⁰ (1927) in a study of 109 cases of rheumatic disease of the heart, noted that the myocardium was involved in 8 cases where the valves and pericardium were normal. VonGlahn and Pappenheimer¹¹ (1926) have published data of 2 of these cases.

CASE 1: Male, aged 68 years. There was no history of tonsillitis, arthritis or chorea. Wasserman reaction negative. Past history suggested myocardial insufficiency. Autopsy showed acute rheumatic myocarditis with Aschoff bodies present, thrombi in the right auricle and the right and left ventricles, emboli in the right internal carotid and right middle cerebral arteries, infarct of the brain and rheumatic lesions in the blood vessels of the testes and pancreas.

CASE 2: Female, aged 33 years. There was a history of tonsillitis but no history of arthritis, chorea or cardiac trouble. Autopsy revealed rheumatic myocarditis with Aschoff bodies present, rheumatic lesions in blood vessels of the ovary and kidney, chronic nephritis and hypertrophy of the heart.

*Chiori's Case*¹² (1928): Female, aged 29 years. There was a history of "heart trouble" over a period of several years and of shifting pain in the joints beginning 6 months before death. Death was due to heart failure. Autopsy showed a fresh fibrinopurulent pericarditis, cloudy swelling of myocardium, liver, and kidneys, and acute splenic tumor. Microscopic examination revealed Aschoff bodies in the myocardium and characteristic rheumatic lesions in the aorta.

Of considerable importance are the findings of Swift,¹² Holsti,¹⁴ and Kugel and Epstein¹⁵ that valvulitis may be present in leaflets that grossly appear normal or little altered. One can only speculate

as to the possibility of microscopic changes having been present in the valves in some of the cases reviewed above. Valvulitis is probably early accompanied by slight gross thickening of valve leaflets, and it is not likely that such thickening would be overlooked by one making a diagnosis of rheumatic myocarditis when verrucae were absent from all the valves.

REPORT OF CASE

Clinical History: H-22483. A Chinese girl, S. H. S., aged 12 years, was brought to the Peiping Union Medical College Hospital on Nov. 30, 1928, complaining of shortness of breath, palpitation of the heart, cough, and swelling of the lower extremities and abdomen. In the past she had frequently had sore throat, but there was no history of pain in the joints, long standing fever or severe illness.

The onset of the present illness began with edema of the ankles in June, 1927. A month later the abdomen became swollen and the patient experienced palpitation of the heart and dyspnea. Some fever and "pain in the bones" of the extremities were said to have been present at that time. Two long hospitalizations brought only temporary improvement.

Physical examination showed a well developed and well nourished girl, dyspneic and slightly cyanotic. Venous pulsation was visible in the neck. The heart was markedly enlarged and there was a well marked precordial heave. A systolic thrill could be felt at the apex. The second sound was louder at the pulmonic than at the aortic area. Pulse rate 90 to 96. Blood pressure 108 systolic and 72 diastolic. The liver was markedly enlarged. There was moderate pitting edema of the legs and ankles, and a suggestive fluid wave in the abdomen was obtained.

The urine contained a faint trace of albumin and a few granular casts. Blood examination showed a mild degree of anemia. Electrocardiogram taken at the time of admission showed normal mechanism.

Seven weeks hospitalization brought moderate improvement in the general condition. Symptoms reappeared, however, under light work at home and the patient returned to the outpatient department. Digitalis was prescribed. By mistake she took an overdose, and 4 days later, on Feb. 8, 1930, she was readmitted to the hospital with auricular fibrillation and signs and symptoms more marked than noted at the previous admission. A few squeaking râles were heard at the back of the chest. The temperature was 39.9° C, but later remained normal or subnormal. The pulse rate was 45 and the rhythm was irregular. Blood pressure 105 systolic and 50 diastolic. The white blood cells numbered 12,650, of which the proportion of polymorphonuclear neutrophils was 49 per cent, lymphocytes 44 per cent, large mononuclears 6 per cent, and eosinophils 1 per cent. In the hospital the patient complained of severe pain in the legs. Salyrgan was given several times with marked transitory diuresis following each injection. On account of auricular fibrillation quinidine was given on February 19. The drug was given in three doses at 1½ hour intervals, the first two doses being 0.1 gm. each, and the third dose 0.3 gm. Twenty minutes after the third dose the patient collapsed and died 6 hours later.

The clinical diagnoses were: rheumatic cardiovascular disease, mitral stenosis and insufficiency, cardiac enlargement, cardiac insufficiency, partial heart block, auricular fibrillation, bronchitis and cardiac failure following quinidine therapy.

POSTMORTEM EXAMINATION

Autopsy was performed 17 hours after death. The body was that of a fairly well developed and fairly well nourished Chinese girl weighing 28 Kg. and measuring 137 cm. in length. The vessels of the conjunctival mucous membranes were overdistended with blood. Thick mucus adhered to the nasal septum. The cervical lymph nodes were readily palpable. The nail beds were blue. The thorax flared slightly at the lower border and the abdomen was rounded and much distended. There was no obvious edema of the lower extremities.

The abdominal cavity contained between 2½ to 3 liters of almost clear, amber-colored, watery fluid. The lower border of the liver extended 3 cm. below the costal margin in the right midaxillary line. The intestines contained but little material.

The heart occupied a relatively large volume of the thoracic cavity. The pericardium surrounded the heart rather tightly and was not thickened. There were no pericardial or pleural adhesions and the surfaces were smooth and glistening.

The heart was greatly enlarged, weighing 360 gm. The right auricle and ventricle were much dilated. Anteriorly, near the apex of the right ventricle, the epicardium exhibited a slightly elevated, grayish white area of fibrous tissue thickening. Projecting from the epicardium of the left auricle was a soft, rounded, thick mass 4 mm. in diameter of smoothly outlined, fibrous tissue growth. A few petechial hemorrhages were noted in the epicardium of the left ventricle. The heart chambers contained a soft purple clot, also a jelly-like, chicken fat clot. Thrombi were looked for carefully in all the heart chambers and were not found. The foramen ovale and the ductus arteriosus were closed. The right auricle was dilated so that the trabeculae carneae were conspicuous; the wall in the areas between the trabeculae was quite thin and was semitransparent. The tricuspid orifice admitted three fingers, and the valve circumference measured 11 cm. The valve leaflets were a little diffusely thickened, but were quite pliable and free from vegetations. The dilatation of the right ventricle was marked, and the ventricle wall

was hypertrophied to a thickness of 4 to 6 mm., being thickest just below the pulmonic valve. The pulmonic leaflets were delicate and the valve orifice measured 7 cm. in circumference.

The left auricle was much hypertrophied and dilated. The auricular appendage was, however, normal in size. The endocardium of the right lateral wall of the auricle showed a corrugated, slightly elevated (about 1 mm.), poorly outlined patch 5 or 6 cm. in area. Except for the presence of a number of small, pinkish red mottlings, the color of the patch did not differ from that of the normal endocardium. Opposite this area the previously noted mass of soft fibrous tissue took its origin from the epicardium. Anteriorly and inferiorly to the patch described, the endocardium was slightly thickened and showed several smooth shallow depressions up to 2 mm. in diameter and 1 mm. deep. Over one small area the auricle wall was thin and fibrous. The mitral orifice was abnormally wide, admitting three fingers, and had a circumference of 12 cm. The valve leaflets and some of the chordae tendineae were a little thickened, but the leaflets were quite flexible and were not shrunken. There were no vegetations or nodular thickenings on the leaflets.

The left ventricle was hypertrophied and moderately dilated so that the apex was rather rounded. The wall varied in thickness from 16 mm. at the base to 11 mm. at the apex. The endocardium was slightly thickened and had a gray color below the aortic valve and over the trabeculae near the apex. The aortic orifice measured 6.5 cm. in circumference. The leaflets were delicate. The myocardium was flabby, and the cut surface had a glistening, light brownish pink color with some delicate mottlings of yellow and pink and a few small, opaque, gray, linear markings. The large coronary arteries were normal in appearance.

Near the sinuses of Valsalva and in the abdominal portion the aorta presented small, delicate, barely visible grayish yellow mottlings. The pulmonary artery and its large branches showed elevated grayish yellow plaques. Iliac, common carotid, splenic and mesenteric arteries and the celiac axis appeared to be normal.

The lungs, particularly the right, were a little heavier and firmer than normal. The large bronchi contained thick mucus and foamy mucoid fluid. Tracheobronchial lymph nodes were enlarged, and their cut surfaces were a velvety reddish black. One lymph node showed grayish areas judged to be tubercles. The lung cut surfaces

were brownish red and pink. Scattered in both lungs, but found more abundantly in the right and especially in the middle lobe, there were small, slightly elevated, dark red, solid areas. Pressure caused the escape of a little foamy fluid from the cut surface of the left lung, and a considerable amount in the case of the right lung.

The liver was much enlarged and weighed 1200 gm. The cut surface showed nutmeg-like, reddish purple markings and in places the lobule architecture could not be made out. The spleen was enlarged to weigh 235 gm., was firm, and presented a pinkish red, slightly velvety cut surface with inconspicuous trabeculae and readily visible malpighian bodies. The intestinal veins contained blood, and the intestinal mucosa was a grayish red color. The stomach mucosa was a dark red color and was covered by thick mucus. Mesenteric and pancreatoduodenal lymph nodes were somewhat enlarged. The kidneys were rather firm and weighed 160 and 140 gm. respectively. The capsules stripped easily, revealing smooth, purplish red surfaces. Cut surfaces were darker red than normal, striations were straight, and malpighian bodies were conspicuous. The cortex of the suprarenal glands was not as yellow in color as normal. The tonsils were small and not remarkable in gross. Paratracheal and deep cervical lymph nodes were a little enlarged, but cut surfaces were not unusual. Most of the supporting soft tissues of the body were abnormally moist.

The brain appeared anemic. In the right carotid artery, at the point of its division to form vessels of the brain, there was an obstructing mass that was not readily removed. Mucosa of sphenoid and maxillary sinuses was a little thickened and abnormally moist. Turbinate mucosa, ethmoid and mastoid cells, and middle ears appeared normal.

MICROSCOPIC EXAMINATION

Tissues were fixed in Zenker-formol and in 10 per cent formalin, and later several blocks were cut from tissues fixed in Kaiserling fluid. Sections were stained with hematoxylin and eosin, except as otherwise noted.

Heart: In four sections through the left ventricle, the muscle striations stain rather poorly, and many of the cells appear shrunken and degenerated. Some fragmentation is present. A scharlach R

stained frozen section reveals only a small amount of fat in the muscle cells, and this is diffusely distributed. In some areas the connective tissue of the epicardium and endocardium and that between shrunken muscle cells contains a few small round cells resembling lymphocytes, some large mononuclear wandering cells, and a few polymorphonuclear leukocytes. In a section taken through the patch of thickened epicardium, noted in gross on the right ventricle, the structure is seen to be composed of loosely woven, highly vascularized connective tissue sparsely infiltrated by small round cells. Another section taken through the right ventricle shows several small areas of muscle cell degeneration with fibrous tissue proliferation adjacent. In the two sections taken the posterior leaflet of the mitral valve shows no lesions.

Five blocks were taken through the left auricle in the region of, or adjacent to, the corrugated patch. The endocardium in all these sections is distorted and thickened by connective tissue growth, and together with the adjacent muscle layer shows wandering cell infiltration and usually edema. In the sections through the corrugated patch, thickening of endocardium by loosely woven connective tissue is marked, and varies in degree to make the surface quite irregular in outline. In certain areas fibroblasts are numerous; in other more edematous areas the young connective tissue cells are undergoing degeneration with vacuolization of cytoplasm and karyolysis. A lining of endothelial cells is recognized over most of the endocardium, but one section shows several areas of old and fresh blood and fibrin in crypts apparently formed by closely approximated folds of thickened endocardium. Numerous large mononuclear wandering cells, numerous small round cells resembling lymphocytes, and a few polymorphonuclear leukocytes diffusely infiltrate parts of the newly formed endocardium and are most abundant in the lower part of the edematous endocardium adjacent to the auricle muscle. Blood capillaries are conspicuous in this lower layer of endocardium and in near-by muscle, and the cellular infiltration is often marked about these vessels. There is also a tendency for the infiltrating cells to be arranged in long, thick and thin rows parallel with the surface, these being apparently lines of separation in the connective tissue. The interstitial tissue of the muscle layer is edematous, and many of the muscle cells are poorly preserved. Hypertrophy of muscle cells is marked. In some areas, especially about blood vessels, muscle cells

have been replaced by loosely woven fibrous tissue, and this tissue holds a few small round cells and mononuclear wandering cells. In several of the sections the epicardium is thickened by edematous fibrous tissue containing dilated capillaries, a moderate number of small round cells and large mononuclear wandering cells. The soft epicardial growth opposite the corrugated patch is seen to be a highly vascularized, edematous growth of fibrous tissue infiltrated by many inflammatory cells of the types just mentioned.

Aschoff bodies were looked for carefully in all the sections but were not found. Some of the large mononuclear cells in the left auricle exudate resemble Aschoff cells in appearance, and with the methyl green-pyronin stain the cytoplasm of a few such cells stains red. The stain could not be said to be satisfactory, however, with Zenker-formol-fixed material. The coronary arteries appear normal. Van Gieson and Mallory connective tissue stains demonstrate well the increase in connective tissue in the auricle endocardium, epicardium and interstitial muscle tissue. Verhoeff elastic tissue stain shows elastic fibers in some of the newly formed connective tissue of the auricle endocardium. With the MacCallum bacteria stain and with the Levaditi stain no organisms are demonstrated.

Aorta: In some of the four sections the intima is a little thickened by loosely woven or moderately dense fibrous tissue. In places the connective tissue cell nuclei are more thickly distributed than in normal intima, and a large mononuclear wandering cell is occasionally seen. Fat stain shows minute fat globules in the cytoplasm of some of the intimal connective tissue cells. Parts of the media appear to be edematous, and there are large linear areas where normal tissue is replaced by delicate connective tissue fibers. Loss of tissue is also evident about some of the vasa vasorum. The Verhoeff-Van Gieson combination stain shows the loss of elastic tissue in parts of the media, and this change is greater in the half of the media nearest the intima. The internal elastic lamella in places shows poorly. In the adventitia of two of the sections, and of one in particular, there is moderate perivascular and diffuse infiltration by large mononuclear wandering cells, a few plasma cells and a few small round cells resembling lymphocytes.

Pulmonary Artery: Sections taken through the grayish yellow plaques show great thickening of intima by loosely woven fibrous tissue, and many cells contain fat. The media appears to be slightly

edematous, the elements being spread apart to a greater extent than normal. The Verhoeff-Van Gieson stain demonstrates diminution of elastic tissue fibers, not only adjacent to the intima but also to a slight extent in other parts of the media. Recognizable, partially disintegrated elastic tissue fibers are seen in some of the linear areas not taking the black stain. The adventitia in one of the sections shows large mononuclear wandering cells and small round cells, perivascularly and diffusely distributed.

Other Vessels: In the pulmonary vein and in the several medium-sized arteries and veins examined, the only thing found worthy of note is slight intimal thickening in the superior mesenteric artery. In the section through the right internal carotid artery just below its point of bifurcation the lumen is filled by a laminated structure composed of poorly preserved fibrin, red blood cells, nuclear débris and material that appears to be disintegrated blood platelets. At the edge of the mass there is well preserved fibrin enmeshing intact blood cells. The mass is not attached to the intima of the artery.

Other Organs: The lung alveolar wall capillaries are dilated, and a little thickened by fibrous tissue. Many alveoli contain coagulated fluid, red blood cells and large mononuclear cells, some of the latter containing much brown pigment. In areas alveoli contain coagulated fluid, fibrin and polymorphonuclear leukocytes. In a section from the left lung a few collapsed alveoli are present. The mucosa of a main bronchus contains a moderate number of lymphocytes and a few polymorphonuclear eosinophils. In a bronchial lymph node blood capillaries are conspicuous and a few epithelioid cell tubercles are to be seen; in another section the sinuses contain a few red blood cells and large mononuclear wandering cells with a polymorphonuclear leukocyte occasionally visible.

The sinuses of the spleen and the liver are greatly distended with blood. The splenic connective tissue is slightly and generally increased. Liver cells about many of the efferent veins are atrophied, and contain fat and bile pigment. Half a dozen well formed miliary tubercles are noted.

In the kidneys the capillaries are tremendously dilated. Many capsular spaces contain finely granular material and there appear to be fibrin adhesions between a few glomeruli and their capsules. The tubule epithelium stains poorly and exhibits swelling, partial disintegration and karyolysis. Many tubules contain hya-

line and mononuclear cell casts, and finely granular material. A small amount of fat can be demonstrated in the tubule cells. Interstitial connective tissue is increased diffusely to a slight extent, and to a considerable extent in some areas where leukocytes are present. In sections of stomach and intestine blood capillaries are greatly dilated. No parasites or ova are found in the intestinal contents. Cortical cells of the suprarenal glands contain but little lipoid. No *Treponema pallida* are found in a Levaditi-stained section of suprarenal gland.

The tonsils show small fibrous tissue scars and numerous dilated crypts containing epithelial cell debris and masses of bacteria. Polymorphonuclear leukocytes are found in a few crypts and also in their epithelial cell walls. The MacCallum bacteria stain reveals in the crypts numerous Gram-positive cocci, often in chain formation. In the large masses of organisms, bacilli of several types outnumber the cocci. In two sections from nasal sinuses the mucosa is edematous and infiltrated to a greater extent than normal with lymphocytes and larger mononuclear wandering cells. Some polymorphonuclear leukocytes are present. Gram-stained smears from the sphenoid sinus show a few polymorphonuclear leukocytes and numerous Gram-positive cocci in pairs and chains. Sections of thyroid, thymus, parathyroid, hypophysis, ovary, uvula, esophagus, turbinates, urinary bladder, pancreas, voluntary muscle, femur bone marrow, and brain present nothing particularly noteworthy.

Bacteriological Examination: Culture of heart's blood in broth, and cultures of heart's blood and of spleen tissue on blood agar plates showed no growth after 48 hours incubation.

Anatomical Diagnoses: Rheumatic fever with lesions in endocardium (left auricle), myocardium, epicardium, aorta and pulmonary artery; cardiac hypertrophy and dilatation; fibrous epicardial plaques; mitral insufficiency; arteriosclerosis of pulmonary artery; marked chronic passive congestion of viscera; ascites; anasarca; edema of lungs (slight); partial atelectasis (left). Chronic bronchitis; early lobular pneumonia; chronic lymphadenitis of bronchial, tracheal and cervical lymph nodes. Hyperplasia of mesenteric lymph nodes. Embolus in right internal carotid artery. Slight subacute nephritis. Chronic tonsillitis. Slight sphenoid and maxillary sinusitis. Tuberculous lymphadenitis of bronchial lymph node; miliary tubercles in liver. Accessory spleen.

DISCUSSION

The absence of Aschoff bodies in this case does not preclude the diagnosis of rheumatic fever. In 190 cases of rheumatic fever reviewed by Clawson¹⁶ Aschoff bodies were found in only 67 per cent of the cases. Thayer's¹⁷ included series of 24 cases showed the highest reported incidence of Aschoff bodies, 87.5 per cent. In 24 picked, active cases Kugel and Epstein¹⁵ reported Aschoff bodies in 79.2 per cent; and in 28 picked active cases McClenahan and Paul¹⁸ reported Aschoff bodies in 85 per cent. The last two sets of figures offer additional support to Clawson's statement that "the more acute the disease process the greater is the likelihood of Aschoff bodies being present." The case reported here was chronic in character.

The lesion in the left auricle in this case was the most important single piece of evidence leading to the diagnosis of rheumatic fever. MacCallum¹⁹ first described the auricle lesions characteristic of this disease. VonGlahn²⁰ confirmed the finding, and reported characteristic auricle involvement in 9 of 31 cases examined. In Thayer's series¹⁷ of 25 cases the left auricle was involved in 10 cases. Kugel and Epstein¹⁵ record auricle lesions in 16 of 24 cases. When these figures are combined there is record of auricle involvement in 35 of 80 cases (44 per cent). Clawson¹⁶ reports: "Microscopic evidence of infection in the auricle was found almost constantly."

Characteristic changes in the aorta in rheumatic fever were first recognized by Klotz.²¹ Pappenheimer and VonGlahn^{22, 23, 24} described and illustrated several stages of the lesions in all three layers of the aorta. Perla and Deutch²⁵ added the description of an additional acute lesion consisting of fibrin plaque formation in the intima. In 77 cases examined in which there was presumptive evidence of recent or old rheumatic infection, Pappenheimer²⁶ reported triangular or flame-shaped scars in addition to acute lesions in 40 per cent of the cases. Kugel and Epstein¹⁵ recorded 5 cases with aortic involvement (diffuse inflammatory reaction in the media) in a series of 24 cases of active rheumatic fever. Chiari¹² reported characteristic adventitial changes (with normal media) in 5 of 6 cases where death was due to an acute exacerbation of rheumatic fever, and expressed doubt as to whether the 6th case was really rheumatic fever, inasmuch as Aschoff bodies were not found. Giraldi²⁷

found characteristic microscopic lesions of the aorta in all of 5 consecutive cases of acute rheumatic fever. Klinge and Vaubel²⁸ described the aortic changes in a series of cases of rheumatic fever and recognized three stages in the life history of a lesion.

The changes of the aorta in the case here reported do not fit the picture of syphilis or of medionecrosis aortae, as described by Gsell, by Erdheim, and by Cellina. Aortitis occurs in various diseases in which bacteria circulate in the blood — focal reaction, necrosis and thrombosis are features of such — but, as Siegmund²⁹ has shown, the picture is not necessarily completely developed. Necrosis of media has been described in infectious diseases, notably in scarlatina, diphtheria and typhoid fever. Because the aortic lesion in this case was in a rather inactive stage and showed no specific cellular reaction, one should be open-minded with regard to its interpretation. However, considering all the facts in the case, the lesion is best explained as being related to rheumatic fever. As in the first cases described by Pappenheimer and VonGlahn²² and a case more recently described by Gray and Aitken³⁰ (Case 3), this case showed scarring of the media as the most striking alteration. The cellular infiltration and the fibrous tissue thickening of the intima were slight but perfectly definite.

Lesions similar to those found in the aorta in rheumatic fever have been described in the pulmonary artery by Paul,³¹ Kugel and Epstein¹⁵ (5 instances in 24 cases), Gray and Aitken,³⁰ Shaw,³² and Chiari.³³ In the case here described there was moderate cellular infiltration in the adventitia of the pulmonary artery, the media appeared to be edematous and showed degeneration of elastic tissue fibers. Because of the marked arteriosclerotic change in the intima one probably is not justified in emphasizing the rheumatic character of the rather slight lesions in the media, but they fit in with the diagnosis of rheumatic fever as made on the basis of other evidence in the case.

The heart was hypertrophied and greatly dilated to the extent that beriberi was at first considered as a possibility. The mitral ring was enlarged to produce a relative mitral insufficiency. Long-standing passive congestion of the pulmonary circulation was evidenced by the arteriosclerosis of the pulmonary artery and by the fibrosis and pigmentation of the lungs. Marked chronic passive congestion of abdominal viscera, ascites and anasarca were signs of myocardial

insufficiency. The degree of myocarditis of the ventricles was not great, but there were moderate diffuse fibrosis and degenerative change in the muscle. In the left auricle the endocarditis and myocarditis were marked. Was there at one time a slight valvulitis of the mitral leaflets? If so, no gross or microscopic evidence remains to prove it. Scars in the auricle extend rather close to the valve ring. The slight thickening of the mitral leaflets and of some of the chordae tendineae could be related to the increased strain to which they must have been subjected because of the imperfect closure of the valve. The autopsy findings correlate well with the history that the first signs of congestive heart failure appeared 1 year and 8 months before death.

The embolus in the right internal carotid artery explains the mode of death. Such an occlusion would not be fatal in an individual with good blood circulation, but it would be expected to be so in one having a decompensated heart. Although no thrombi were found in the heart, the presence of disintegrating blood and fibrin in the endocardial lesion of the left auricle makes it the likely site of thrombus formation. It is interesting to note that Willius,³⁴ in an analysis of the mode of death in 160 cases of rheumatic heart disease, reported death from cerebral embolism in 4 cases, and one other death from coronary occlusion by an embolus derived from a thrombus in the left ventricle. There were thrombi in the auricular appendages in 5 of Thayer's¹⁷ 25 cases. In 1 of the above noted cases of Von Glahn and Pappenheimer¹¹ without valve lesions death was due to cerebral embolism. It is evident that cerebral embolism may be expected occasionally as a terminal event in rheumatic heart disease. In our case the short time between the administration of quinidine and death leads to the belief that quinidine effect was responsible for dislodging a thrombus from the auricle. Eismayer³⁵ collected records of 12 instances of embolism in 934 cases of heart disease treated by quinidine; 16 other deaths in his series were apparently due to the direct effect of the drug.

SUMMARY

In the literature there have been noted 17 cases in which the diagnosis of rheumatic fever was made at autopsy and in which all the heart valves were apparently free from rheumatic lesions; all of these

cases exhibited myocarditis with Aschoff bodies present, and in 4 cases pericarditis also was present.

Based on criteria as we know them at the present time, there is reported an additional case of chronic rheumatic fever in which the heart valves were free from rheumatic lesions. Aschoff bodies were not found and the diagnosis of rheumatic fever depended on the presence of characteristic lesions in the left auricle, scars in the media of the aorta, slight changes in the media of the pulmonary artery, and diffuse inflammatory cell infiltrations in the heart and in the adventitia of the aorta and the pulmonary artery. Death came 1 year and 8 months after the first symptoms of congestive heart failure, following the administration of quinidine. An embolus, accounting for the mode of death, was found in the right internal carotid artery and is believed to have been formed on the rheumatic endocardial lesion in the left auricle.

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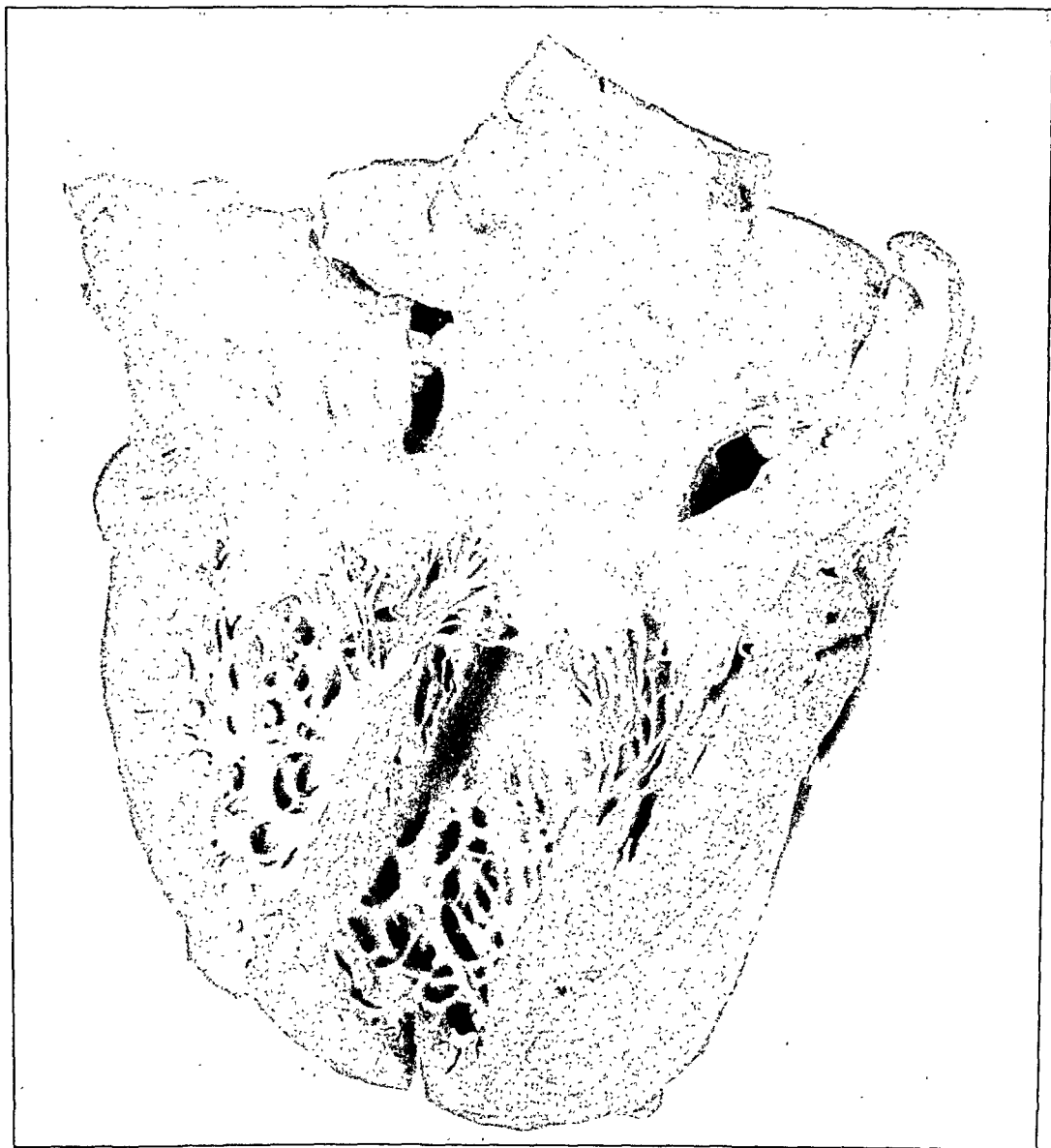
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DESCRIPTION OF PLATES

PLATE 68

FIG. 1. Heart. Hypertrophy and dilatation are apparent. Corrugated areas of thickened endocardium are visible near the lateral edges of the auricle, on the right side of which are a number of crater-like scars of healed endocardial lesions. The auricle wall near-by is thin. The mitral ring is enlarged to produce relative insufficiency of the valve. Except for slight thickening the valve leaflets are normal. Small, polypoid fibrous tissue growths projecting from the auricle are visible in the upper left-hand corner of the photograph.



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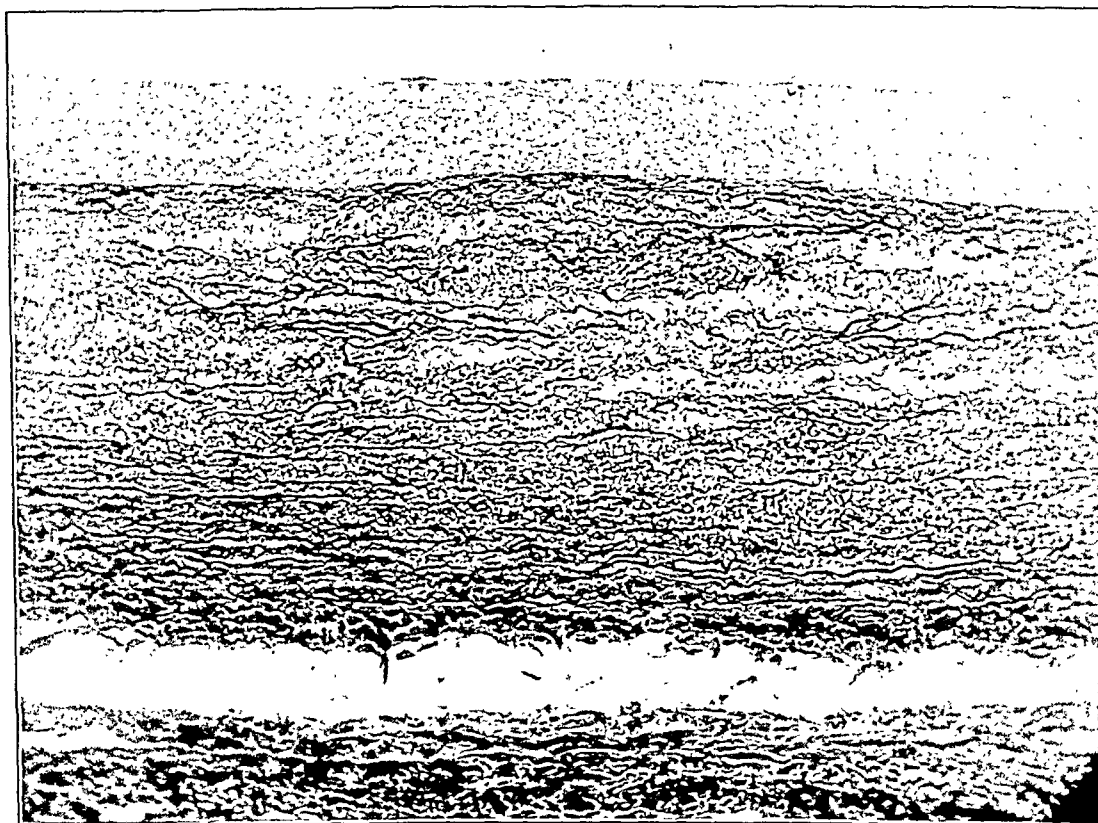
PLATE 69

FIG. 2. Left Auricle. Hematoxylin and eosin stain. The section was taken through the corrugated patch and shows irregular fibrous tissue thickening of the endocardium, a great number of infiltrating inflammatory cells (visible merely as black dots), some fibrosis of the muscle layer and slight thickening of the epicardium. The two dark patches in the endocardium at the upper left represent areas of thrombus formation. $\times 15$.

FIG. 3. Aorta. Verhoeff-Van Gieson stain. The elastic tissue fibers of the media are much diminished and the media architecture is distorted. The intima is a little thickened by fibrous tissue. $\times 90$.



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THE PERCENTAGE OF THE DIFFERENT TYPES OF CELLS IN THE ANTERIOR LOBE OF THE HYPOPHYSIS IN THE ADULT HUMAN FEMALE *

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INTRODUCTION

The necessity of greater accuracy in the evaluation of the relative number of the different types of cells regularly recognized in the anterior lobe of the hypophysis was briefly discussed in a previous report¹ that dealt with a differential count of these cells in 111 normal male adults. The emphasis recently placed on basophilic adenomas of the pituitary (Cushing,² Moehlig³ and others), and the suggestion that profound abnormalities (painful adiposity, kyphosis, sexual dystrophy, and so on) may result from comparatively small masses of basophilic cells, serve further to emphasize the point.

Acidophil and *basophil* are used instead of the more exact terms *alpha* and *beta*, proposed by Bailey and Davidoff,⁴ because of more common usage. Other designations for these chromophilic cells, as well as for the chromophobes, will be found in a preceding article.¹ The section on the hypophysis by Bucy,⁵ recently published, may further be cited for the normal cytology.

On account of the close interrelations of this organ with the gonads, a sex difference in the histological structure is of some interest. It has been recognized for some time that in some animals (rat, rabbit, cattle), as well as in the human, the female has a larger hypophysis, due to a larger anterior lobe. Further corroboration of this for the human on the basis of quantitative determinations on over 200 selected cases will be published in the near future. There are, however, no very reliable data showing which of the elements in the anterior lobe is responsible for this difference in size. A recent examination of serial sections of 235 normal, adult human hypophy-

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ses⁶ indicates that gland-like extensions of pars intermedia into the neural lobe is much more frequent in the female than in the male. The incidence of concretions resembling "brain-sand" in the pial investment of the upper aspect of the normal hypophysis was found to be nearly twice as high in females as in males. Neither pregnancy nor age seems to have any relation to the frequency of these two structural details.

Much has been written on the development of a special cell in the anterior lobe of the hypophysis during pregnancy in the human and in certain other animals. Descriptions of these so-called "pregnancy cells" are so contradictory and vary so much (Severinghaus, Engle and Smith⁷ and Stein⁸) that considerable attention has been paid to the identification of such cells in cases of sudden and accidental death of pregnant females. Interest along this line has been heightened by the apparent overproduction of gonad-stimulating hormones by the anterior lobe of the hypophysis during pregnancy (the basis of the Aschheim-Zondek⁹ test for pregnancy).¹⁰ While one might anticipate under these circumstances that there would be recognizable histological changes having a bearing on the specific function of the different types of cells, it must also be remembered that cells that are apparently similar morphologically, if not actually identical, may produce several hormones, just as the cells of the pancreatic alveoli, for example, produce several ferments.

These considerations are the justification for the establishment of norms toward which it is hoped these data will contribute.

METHOD

The histological technique has been uniform throughout and is the same as that used on the male hypophysis.¹ It consists essentially of formalin fixation and staining, first slightly with hematoxylin (to bring out the nucleus, which is important in counting cells so variable in size), and then (after washing in tap or alkaline water followed by distilled water) in Mallory's connective tissue stain (acid fuchsin followed by a mixture of orange G and anilin blue) as in the usual procedure for collagen fibers (Mallory¹¹), with the precaution that the orange G-anilin blue stain must be washed off in not lower than 95 per cent alcohol and dehydrated rapidly in absolute alcohol. To bring out most strikingly the basophilic cells, omit the acid fuchsin

and stain only in the orange G-anilin blue mixture. By leaving out the acid fuchsin differentiation between the acidophilic cells and the chromophobes is not so marked. This is a far simpler technique than that proposed by Bailey ¹² and adequate for general purposes where mitochondria and other finer cytological features are not required. The colored frontispiece of Cooper's book ¹³ is no exaggeration of the brilliancy obtainable, even on material obtained twelve or more hours after death. It works fully as well after saturated corrosive sublimate containing 10 per cent formalin, and Howes ¹⁴ finds it the most effective stain, particularly after Gilson's fixation (a corrosive sublimate mixture). If neutral formalin is used as the original fixing agent and either the block or the sections mordanted in potassium dichromate, finer cytological details may be brought out by mitochondrial and other stains. As Bailey ¹⁵ again points out, the hematoxylin and eosin stain is not differential enough.

If such a small tumor as was present in the Bauer case (cited by Cushing²) is of as much consequence as that and some other cases suggest, it is to be hoped that in all suspicious cases of hypophyseal involvement, whether there are any gross indications in the hypophysis or not, the complete gland will be sectioned serially and thirty or more sections at equal intervals stained. If cut 10 microns thick, every twentieth section is enough. These can usually be mounted on four ordinary slides and hence involve very little extra work. For quantitative evaluation of the number of cells thinner sections are necessary.

Kernohan ¹⁶ has found that ordinary formalin-fixed human hypophysis may be treated easily so as to stain differentially also by Heidenhain's modification of Mallory's stain. The basophils are very prominent by this method. Biedermann ¹⁷ used still another combination of stains that differentiates well the three types of cells after formalin-fixation, and Soós and Csizek ¹⁸ have recently reported that a resorcin-fuchsin stain is highly selective for the basophilic granules, also on formalin material. Since formalin is always available at post-mortem examinations and permits the use of so many differential stains, it would seem to be the best fixation for purposes of routine examination.

For certain animals and for specific purposes some highly differential techniques have recently been devised by Cleveland and Wolfe ¹⁹ and Severinghaus.²⁰ Gathering material for this study commenced

thirteen years ago, and in order to make results comparable the same method has been followed throughout. These newer methods have therefore not been used for the quantitative work on this human material. It may not be amiss to call attention also to a paper by Weiss,²¹ recently published, which further broadens the application of Mallory's connective tissue stain in general histological technique.

Specimens were taken at autopsy by carefully pinching off the sella turcica with heavy bone forceps, leaving the hypophysis *in situ*, and putting the entire mass immediately in formalin and transporting it to the laboratory where the hypophysis was dissected out.* The dura and another very thin layer of connective tissue, representing the arachnoid membrane, were carefully removed except from about the posterior lobe, where these meninges are so adherent that it is inadvisable to attempt to remove them if the posterior lobe is to be kept intact. Only in cases of sudden and usually accidental death, where there were no evident hypophyseal involvements, was material used.

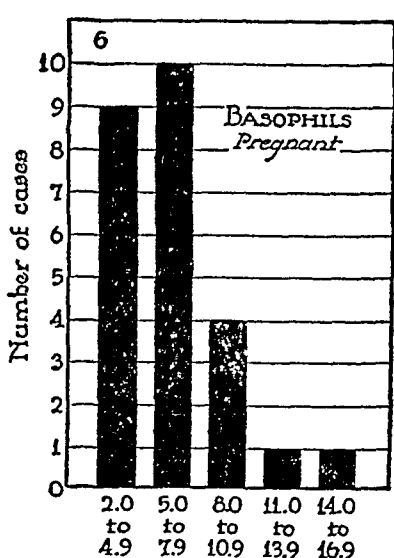
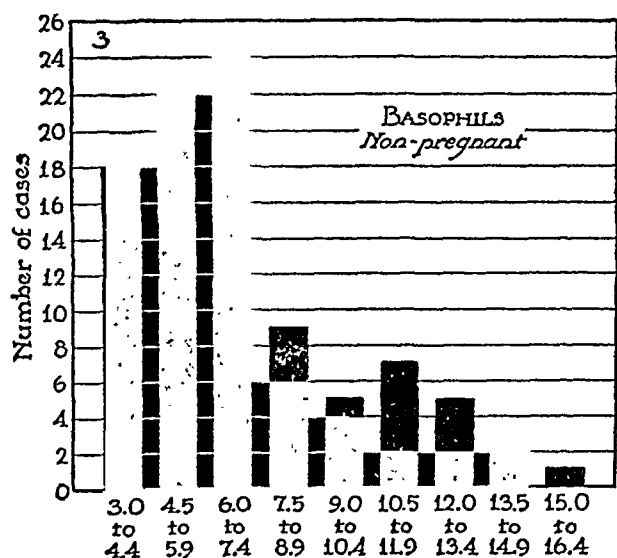
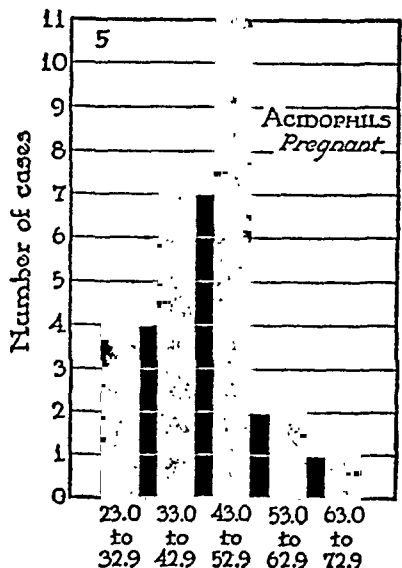
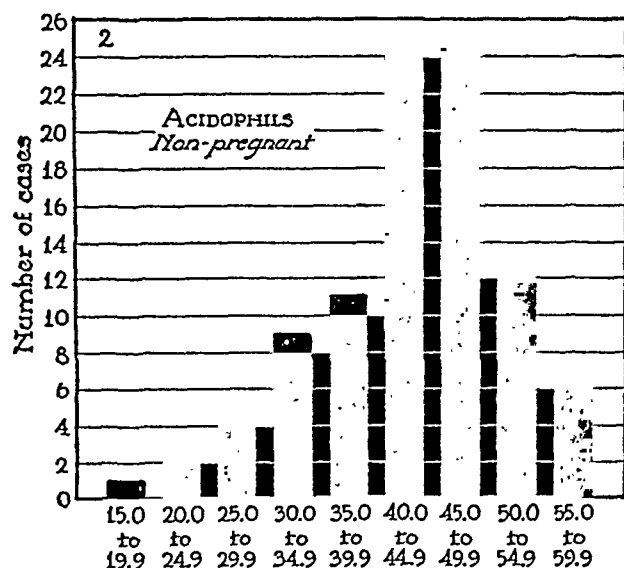
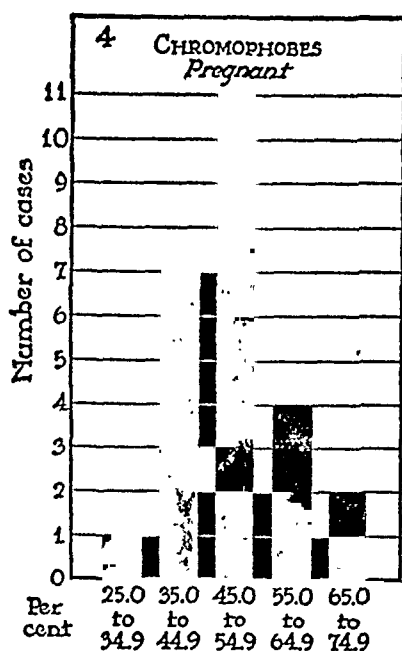
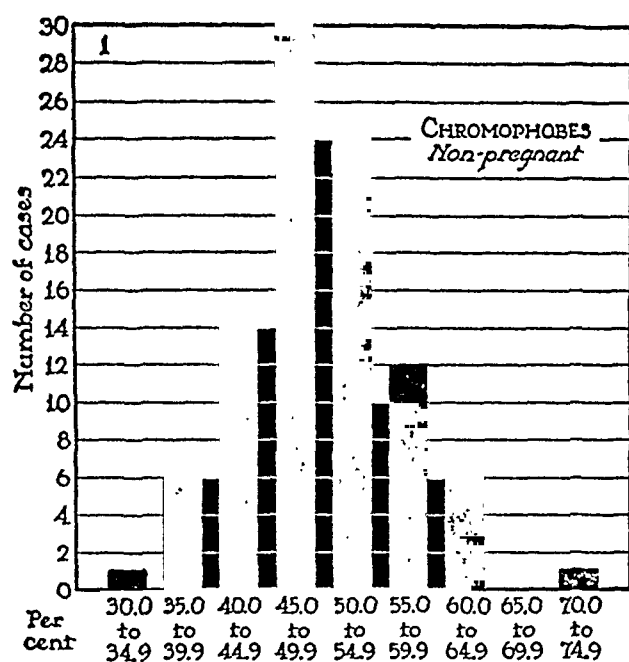
The results have been tabulated by means of the commoner statistical methods, which are necessary for properly evaluating differences, especially where these are relatively small and the number of cases limited. Lack of statistical constants and details of methods used render much published data almost valueless for scientific purposes.

RESULTS

Frequency Distribution: The distribution of the percentages of the three types of cells of 94 non-pregnant and 25 pregnant females is shown graphically in Charts 1 to 6. These graphs and subsequent tables are based on an average of 214 sample fields and an average total, in round numbers, of 15,000 cells in each hypophysis (about 70 cells per field of oil immersion lens). The basophils are distinctly skewed in the positive direction, as is also the case in the male¹; but otherwise there is the usual tendency to form a fairly symmetrical curve.

Percentage of Chromophobes in Non-Pregnant Females: Table I shows that the chromophobes vary from 33 per cent to 74 per cent and average between 49 and 50 per cent. The coefficient of variation

* For the coöperation of various members of the Department of Pathology of the University of Minnesota, and especially Drs. J. S. McCartney and B. J. Clawson, in securing specimens, the author is greatly indebted.



CHARTS 1-6

Frequency distribution of the percentage of the different types of cells in the anterior lobe of the hypophysis of 94 non-pregnant and 25 pregnant females.

is 14, which is of the same order as the variability of the weight of the normal liver.

Percentage of Acidophils in Non-Pregnant Females: The upper and lower limits (Table I) are about 15 per cent lower than the chromophobes, but the average (about 44 per cent) is only 6 per cent less. The coefficient of variation is 19. This is slightly greater than the variability in the normal weight of the heart.

Percentage of Basophils in Non-Pregnant Females: The basophils (Table I) show a relatively greater individual variation, ranging from 3 to nearly 16 per cent, with an average of 7 per cent. The co-

TABLE I

*Percentage of the Different Types of Cells in Anterior Lobe of Human Hypophysis
(94 Normal, Non-Pregnant Females)*

Cell type	Minimum	Maximum	Median	Mean and prob. error	Standard deviation	Coefficient of variation
Chromophobe . . .	32.9	74.1	49.0	49.6 ± 0.47	6.79	14
Acidophil	19.2	57.5	44.2	43.4 ± 0.56	8.10	19
Basophil	3.0	15.6	6.6	7.0 ± 0.20	2.94	42

efficient of variation 42 means that they are nearly as variable in relative number as the thyroid varies in weight, the corresponding constant for the thyroid gland being 45.

Percentage of the Different Types of Cells with Age: Table II shows that when the 33 cases above 50 years of age (average 61 years) are

TABLE II

Mean Percentage of the Different Types of Cells in the Anterior Lobe of the Hypophysis According to Age (Non-Pregnant Females)

Cell type	50-84 yrs. (33 cases)	16-49 yrs. (61 cases)	Difference	P.E. of difference	Difference P.E. of difference
Chromophobe . . .	52.2 ± 0.81	48.2 ± 0.54	+4.0	0.97	4.1
Acidophil	39.6 ± 0.93	45.5 ± 0.64	-5.9	1.13	5.2
Basophil	8.1 ± 0.37	6.4 ± 0.22	+1.7	0.43	4.0

compared with those under 50, whose average age is 31 years, the chromophobes are 4 per cent and the basophils nearly 2 per cent higher and the acidophils 6 per cent lower in the older group. The

last column indicates the ratio of the differences to the probable error of these differences. Since in each case this ratio is 4 or more, this is statistically significant. A ratio of 4 means that there is less than one chance in a hundred that the difference is merely due to random sampling. The same was true in the male, except there the increase in the basophils with age was too small to be significant. The prevalent idea one obtains from the literature is the same as indicated in these females.

Relation of Types of Cells to Stature: The average stature of the 94 non-pregnant females was 162.2 cm. (median 162 cm.) and of the 25 pregnant females 162.7 cm. (median 162 cm.). This is very close to the average stature for this region.²² A comparison of those below the average with those above the average is found in Table III, with a test of the significance of the differences. The slight differences are

TABLE III

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Non-Pregnant Females Below Average Stature with Those Above Average Stature

Cell type	Stature 163-178 cm. (45 cases)	Stature 136-162 cm. (49 cases)	Difference	P.E. of difference	Difference
					P.E. of difference
Chromophobe . . .	50.4 \pm 0.74	48.8 \pm 0.60	+1.6	0.95	1.7
Acidophil.	42.3 \pm 0.86	44.4 \pm 0.73	-2.1	1.13	1.9
Basophil.	7.2 \pm 0.25	6.8 \pm 0.31	+0.4	0.40	1.3

not significant. To be significant the ratios in the last column should be at least 3. In spite of the pathological and experimental evidence for the close association of the acidophils with growth processes, there is no suggestion here along that line, confirming the previous report on the male.¹

Effect of Pregnancy on the Relative Number of the Different Types of Cells in the Anterior Lobe of the Hypophysis: There is much disagreement in the statements concerning the histological changes found in the hypophysis during pregnancy.^{7, 8} A fairly common notion is that there is increased acidophilia, either due to an increase in the number of acidophils or the development of a new cell from chromophobes — the so-called “pregnancy cell” — which in the human is said to be more or less acidophilic in staining properties, so much so that Bailey²³ speaks of it as a *beta* cell. There are, however, others who

regard it as more like a chromophobe.²⁴ Biedermann,¹⁷ after a special study of the tinctorial reaction of these cells, decided that they are enlarged chromophobes with slight basophilic properties.

With the technique and material here involved and attempts to chromate sections from the formalinized tissue and apply the staining method of Cleveland and Wolfe,¹⁹ Kernohan,¹⁶ and other methods, as well as the hematoxylin and eosin stain, no special cell could be found that was characteristic of the hypophysis of females who had been pregnant only a few days before death or who even had a fetus *in utero* when the postmortem was performed. It is true there were only 4 at, or near full term, and that half of them were less than six weeks pregnant, but the rest of the 25 cases occurred in the

TABLE IV

Percentage of the Different Types of Cells in Anterior Lobe of the Hypophysis of 25 Pregnant Females

Cell type	Minimum	Maximum	Median	Mean and prob. error	Standard deviation	Coefficient of variation
Chromophobe . . .	26.6	71.0	49.6	50.1 \pm 1.26	9.33	19
Acidophil.	23.3	64.5	45.7	43.4 \pm 1.34	9.91	23
Basophil.	2.1	14.3	5.7	6.3 \pm 0.51	3.04	48

intervening months, so that there should be some evidence of any marked departure from the appearance of the hypophysis in non-pregnant females.

One gains the general impression that possibly the chromophobes have on the average more cytoplasm, as if the smaller ones have hypertrophied to some extent. Extensive measurements of the size of cells would have to be made to verify this, or show conclusively any other definite change in the size of any of the types of cells. Unusually large chromophobes, different from anything that could be found in the hypophysis of non-pregnant females, were not found. While the cell cords in some pregnant females appeared slightly larger, this was not marked enough to constitute a criterion by which the hypophyses of pregnant females could be distinguished from those of non-pregnant ones.

The results are recorded in Table IV. If the cells were decidedly acidophilic in pregnancy they were counted in with ordinary acido-

phils. On the other hand, if they were like chromophobes tinctorially, they were included with the chromophobes. There seems to be no essential difference between these cases where pregnancy was involved and the non-pregnant ones (Table I). The coefficients of variation are somewhat greater in the group of pregnant females, as might be expected from the small number of cases. How they compare with the figures from a similar age group of 61 non-pregnant females is presented in Table V. There are apparently 2 per cent less acidophils and a corresponding increase in chromophobes in the group of pregnant females, which falls in line with the report of Biedermann¹⁷ and some others, including the recent studies of Wolfe and Cleveland,²⁴ but the ratio of these differences to the probable error

TABLE V

Comparison of the Mean Percentages of the Different Types of Cells in the Anterior Lobe of the Hypophysis in Pregnant Females and Non-Pregnant Females Under 50 Years of Age

Cell type	Pregnant group 15-39 yrs. (25 cases)	Non-pregnant group 16-49 yrs. (61 cases)	Difference	P.E. of difference	Difference
					P.E. of difference
Chromophobe . . .	50.1 \pm 1.26	48.2 \pm 0.54	+1.9	1.37	1.4
Acidophil.	43.4 \pm 1.34	45.5 \pm 0.64	-2.1	1.48	1.4
Basophil.	6.3 \pm 0.51	6.4 \pm 0.22	-0.1	0.56	0.2

of the differences is only 1.4, and hence the differences are not significant. A comparison of the cases in late stages of pregnancy with those in early stages similarly shows no significant differences, but the number of individuals is too small to warrant any definite statement on this particular point.

From this it is evident that the enlargement of the anterior lobe of the hypophysis during pregnancy (most marked during advanced stages²⁵ — confirmative quantitative data on these cases will be published later) is not due to hyperplasia of any particular type of cell. The largest hypophyses in cases of pregnancy in our collection (over 1 gm. in weight) appear distinctly edematous, rather than hyperplastic.

Sex Differences in Cell Proportions in the Anterior Lobe of Adult Human Hypophysis: In Table VI are shown the averages of 100 supposedly normal males (from previous publication¹) and of the 94

non-pregnant females, with a test of the significance of the differences. There are nearly 3 per cent less chromophobes and 4 per cent less basophils in the female than in the male, with a corresponding (6 to 7 per cent) larger number of acidophils in females. These differences are strictly significant, as judged by the ratios in the last column of Table VI.

TABLE VI

Comparison of the Mean Percentages of the Different Types of Cells in the Anterior Lobe of the Hypophysis in Males and Non-Pregnant Females

Cell type	Males (100 cases)	Non-pregnant females (94 cases)	Difference	P.E. of difference	Difference
					P.E. of difference
Chromophobe . . .	52.2 \pm 0.54	49.6 \pm 0.47	+2.6	0.72	3.6
Acidophil	36.8 \pm 0.52	43.4 \pm 0.56	-6.6	0.76	8.7
Basophil	10.9 \pm 0.25	7.0 \pm 0.20	+3.9	0.32	12.2

If only the cases under 50 years of age are considered, in order to eliminate the changes incident to old age and menopause, the sex differences are equally as marked and statistically significant (Table VII).

Since we have just shown that there is, if anything, a decrease in the proportion of the acidophils during pregnancy, the higher per-

TABLE VII

Comparison of the Mean Percentages of the Different Types of Cells in the Anterior Lobe of the Hypophysis in Males and Non-Pregnant Females Under 50 Years of Age

Cell type	Males 18-50 yrs. (69 cases)	Non-pregnant females 16-49 yrs. (61 cases)	Difference	P.E. of difference	Difference
					P.E. of difference
Chromophobe . . .	51.1 \pm 0.56	48.2 \pm 0.54	+2.9	0.78	3.7
Acidophil	38.1 \pm 0.63	45.5 \pm 0.64	-7.4	0.90	8.2
Basophil	10.8 \pm 0.31	6.4 \pm 0.22	+4.4	0.38	11.6

centage of acidophils in females as compared with males is evidently not due to the factor of pregnancy having been involved at some time or another. Naturally, the next step would be to see at what age period this sex difference arises. Hypophyses from children are much more difficult to obtain, so that unless some coöperative effort is made, this point will remain a question for a considerable time.

SUMMARY

1. The results are given of a determination of the relative number of chromophobes, acidophils (*alpha* cells) and basophils (*beta* cells) in the anterior lobe of the hypophysis of 94 carefully selected and supposedly normal, formalin-fixed hypophyses of non-pregnant females 16 to 84 years of age (average 42 years) from cases of sudden or accidental death, and of 25 pregnant females 15 to 39 years of age (average 27 years).

2. The data were obtained by counting all the cells containing nuclei in an average of 214 equally spaced microscopic fields from three different, well separated sections 5 microns in thickness from each hypophysis, and by using Mallory's connective tissue stain after slight staining with hematoxylin to bring out the nucleus.

3. While the above constitutes a highly differential stain that is very easily applied, attention is called to an increasing variety of methods that are being used in an effort to differentiate sharply between the types of cells in the anterior lobe of the hypophysis.

4. In the group of non-pregnant females the chromophobes average between 49 and 50 per cent of all the cells, with a coefficient of variation of 14; the acidophils average between 43 and 44 per cent, with a coefficient of variation of 19; and the basophils average 7 per cent, with a coefficient of variation of 42. These figures are radically different from those given for a mixed group of females and a group of female dementia praecox cases by McCartney,²⁶ in both of which he reports three times as many basophils and only half as many chromophobes. How much of these differences is pathological, and how much is due to the technique used, cannot be determined for lack of details.

5. Female above 50 years of age (average 61 years) show on an average 4 per cent more chromophobes, nearly 2 per cent more basophils and 6 per cent fewer acidophils than those below 50 years of age (average 31 years).

6. There is no correlation between body length and any particular type of cell.

7. While there are on an average relatively fewer acidophils and more chromophobes in the pregnant females than in the non-pregnant, the differences are too small to be statistically significant.

8. Contrary to the opinion of many, the enlargement of the

anterior lobe of the hypophysis during pregnancy is apparently not due to marked hyperplasia of any one of the three generally recognized types of cells, nor could a special so-called "pregnancy cell" be identified.

9. Females have a distinctly higher proportion of acidophils than males, and males, on the other hand, have a higher percentage of chromophobes and basophils.

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THE RELATION OF HEPATITIS TO CHOLECYSTITIS *

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The relation of inflammatory lesions of the liver to cholecystitis has been a question for many years. The clinical observations of Riedel¹ who noted an enlargement of the right lobe of the liver in cases of chronic cholecystitis must be considered one of the earliest references to the association of these two conditions. Charrin and Roger² first called attention to the fact that the injection of bacteria into the common bile duct produced a cholecystitis that was associated with suppurative lesions of the liver. Later Quincke³ and others also mentioned enlargement of the liver in cholecystitis. It was not, however, until Graham⁴ in 1918 published his studies on biopsies of the liver taken at operation in 30 cases of cholecystitis that the conception of hepatitis as a source of gall-bladder infection received much consideration. Since that time many surgical authorities, both in this country and abroad, have accepted Graham's dictum that hepatitis constantly accompanies and is frequently the source of infection in cholecystitis. Graham described an enlargement of the liver and other gross changes such as adhesions and rounding of the liver edge in 87 per cent of his series of cases. Later, however, he⁵ stated: "We are forced to the conclusion that the actual percentage of noticeable enlargement of the liver is very much smaller than the figure given." He felt that the increase in size was due to edema, and in the acute and subacute cases he noted microscopically the constant presence of a hepatitis consisting of an infiltration of lymphocytes and polymorphonuclear leukocytes in the interlobular connective tissue around the bile ducts and vessels. Mild degrees of fatty metamorphosis and other minor changes in the parenchyma of the liver were also noted. In the cases of chronic cholecystitis he described a picture that resembled biliary cirrhosis and suggested the hepatitis of the acute and subacute cases as a possible etiological factor in the production of this condition. In describing these chronic cases, how-

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ever, he does not mention the presence or absence of biliary obstruction, which is conceded by certain workers — namely Charcot and Gombault⁶ and others — to produce a picture of cirrhosis in experimental animals. McCartney⁷ and MacMahon and Mallory⁸ have shown a similar condition in man following prolonged obstruction of the common or hepatic bile ducts. The biopsies of the liver that Graham⁴ removed from his surgical cases were taken from the edge of the liver, which is an extremely unreliable area from which to judge the condition of the liver as a whole. The picture of cirrhosis is frequently seen in this region even in normal livers.

Three years after Graham's original contribution Peterman, Priest and Graham⁹ postulated a lymphatic theory for the etiology of cholecystitis. Basing this theory on the work of Sudler,¹⁰ who showed an intimate connection between the lymphatics of the gall-bladder and extra- and intrahepatic lymphatics, they expressed the opinion that, at least in a certain percentage of cases, infection reached the liver through the portal blood where it produced an inflammatory lesion, and that the infection was conducted from the liver by the lymphatics to the gall-bladder. They quoted Mall's work¹¹ on the lymph channels of the liver, pointing to the fact that since the only demonstrable lymph vessels of the liver substance were situated in the portal spaces, this area would naturally be the first to show evidence of such an infectious process. After the establishment of an infection in the gall-bladder they felt that a retrograde lymphatic infection of the liver from the gall-bladder might keep up a "vicious cycle" that would necessitate the removal of the gall-bladder to effect a clinical cure. In support of this theory they did certain experimental work on dogs. The animals were subjected to preliminary laparotomies and biopsies of the livers were taken and studied. At the same time the cystic ducts and cystic arteries were ligated and the animals were given intravenous injections of 24 hour cultures of non-hemolytic streptococci or *B. coli*. These animals were killed at the end of 19 days and their livers and gall-bladders were studied. The livers that had been normal, as proved by the original biopsies, showed a hepatitis that was most pronounced in the region of the gall-bladder bed. Later Graham and Peterman¹² continued this line of experimentation but varied it by giving one or two injections of bacteria into the portal vein, with much the same results.

Peterman¹³ in 1923 reviewed the whole subject of gall-bladder infection, repeated many of the procedures previously done by himself and other experimenters and in addition he injected bacteria into the appendiceal veins of dogs and noted not only the previously mentioned hepatitis but also a pancreatitis. In his book, published some years later, Graham⁵ described a similar hepatitis in patients operated upon for chronic appendicitis, but was unable to explain why these patients showed no evidence of gall-bladder infection.

The whole lymphogenous theory of cholecystitis advocated by Graham is based on the work of Sudler.¹⁰ Sudler claimed an intimate lymphatic connection between the liver and gall-bladder. Winkenwerder,¹⁴ however, using more refined technical methods, recently came to the conclusion that in the cat there was no lymphatic connection between these organs and so raised some question as to Graham's original thesis.

Graham's ideas, however, have been widely accepted and his work has received confirmation from many sources. Judd,¹⁵ and Judd and Marshall,¹⁶ Deaver,¹⁷ Moynihan,¹⁸ and Heyd, MacNeal and Killian,¹⁹ using Graham's original methods of investigation have largely confirmed his work. Deaver believed, however, that the original lesion occurred in the gall-bladder and that the liver was secondarily involved. Tietze and Winkler,²⁰ studying biopsies of the liver from operative cases, took their specimens from the dome of the liver to eliminate errors that might creep in from examination of the liver edge. They also agreed with Graham. Mentzer,²¹ in a study of 612 consecutive autopsies, showed that the frequency of hepatitis practically paralleled the occurrence of cholecystitis. Martin,²² however, without any stated clinical or experimental data reasoned that the inflammatory reactions in the liver described by Graham were probably secondary to tissue irritation resulting from the absorption of bacteria and toxic substances from the gastrointestinal tract. Aiello,²³ in a postmortem study of 43 individuals dead of infectious diseases, found only 6 instances of definite inflammatory changes in the portal spaces of the liver, and in a series of 14 cases of phthisis he found similar but less marked reactions. He believed that biliary tract infection was not a necessary accompaniment of cholecystitis.

The following study was attempted in an effort to determine how frequently the inflammatory lesions of the liver described by Graham

occur in autopsied cases, and what relation, if any, this lesion bears to inflammatory conditions of the gall-bladder. A total of 212 autopsies was studied. These were unselected but not consecutive. At first it was thought desirable to exclude the autopsies on newborn infants and young children, but as the work developed it became evident that a study of all age groups was indicated, so that 15 cases under 1 year of age and 10 cases between 1 and 10 years of age were included. The work was carried out in the following manner: From each case three blocks of liver and at least one of the gall-bladder were removed. In most instances the tissue from the gall-bladder was taken from the portion of the organ covered by peritoneum, but in a few cases pieces of the gall-bladder were taken through the gall-bladder bed. One section of the liver was always taken from the free margin of the organ, just to the right of the gall-bladder. Two other blocks were removed from the substance of the right lobe well away from the capsule. The tissue was fixed in 10 per cent formalin and stained with hematoxylin and eosin. The gross appearance of the liver and gall-bladder was noted. Only those gross changes of the gall-bladder that were beyond question were recorded in order to simplify tabulation and to exclude non-inflammatory lesions from the group showing cholecystitis. Cholesterosis was not recorded. In the gross descriptions of the liver such changes as chronic passive congestion, increase in fat content, alterations of the capsule and the presence of tumor masses or abscesses were noted. The cases were studied with reference to age and sex, and the cause of death was recorded in each case. The liver and gall-bladder were studied microscopically.

Regardless of gross findings the decision as to whether or not the lesions of the gall-bladder should be called cholecystitis rested on the microscopic examination. Only those gall-bladders showing an infiltration of lymphocytes or leukocytes or both were called cholecystitis. The degree of inflammatory change in the gall-bladder wall was graded as 1, 2, 3 and 4. In Figures 1, 2, 3 and 4 photomicrographs of representative cases in each of the several grades are shown. In the microscopic study of the liver sections three observations are recorded: (1) The degree of infiltration in the portal spaces. This was arbitrarily divided into 4 grades indicated as 1, 2, 3 and 4, and examples of each grade are shown in Figures 5, 6, 7 and 8. (2) The presence or absence of polymorphonuclear leukocytes

was charted and some estimate of their number was attempted by indicating them as "many" or "few." This fact was noted because, especially in the less marked grades of infiltration, the presence of lymphocytes alone is said by some histologists (Maximow and Bloom²⁴) to be a normal occurrence. (3) Varying degrees of cirrhosis were also noted. Most of these were the non-clinical or latent type.

As mentioned above, Graham's^{4, 5} description of hepatitis emphasized particularly periportal infiltration and this lesion alone was studied in this series. Parenchymal changes were disregarded because the variety of pathological lesions causing death produced changes in the liver that had no possible relation to the hepatitis. Tietze and Winkler,²⁰ in their description of hepatitis, stressed changes in the bile ducts such as desquamation of epithelium and the presence of leukocytes in the lumen of the ducts. These changes were not noted by Graham and they were not seen in this series.

The cases were first divided into 10 age groups. Those cases below 1 year of age were placed in one group. The balance of the cases were grouped by decades. All those over 80 years of age were, however, considered together.

In the age group under 1 year the newborn infants were considered separately and the findings are shown in the first portion of Table I. Here, in all cases, a certain amount of infiltration was noted in the portal spaces of the liver. This reaction can hardly be considered as inflammatory in character and a possible explanation is offered by the fact that in all but 1 case myelocytes were seen in the infiltration. The hemopoietic function of the liver is generally considered to be located entirely in the blood islands that are located in the liver lobule. This fact is recognized by Maximow and Bloom,²⁴ and by Downey.²⁵ Nevertheless, the presence of myelocytes in this region indicates the possibility that the portal areas of the liver may also have a hemopoietic function in the newborn.

Most of the remaining cases in this first group, namely those living a period of several months, showed a minimal amount of infiltration in the portal spaces. In the group as a whole 2 Grade 3 reactions were noted, 1 occurring in a newborn infant and the other in an infant dying of primary peritonitis. In the first instance no explanation is offered but in the second case the infiltration was possibly secondary to a general sepsis.

The findings in the gall-bladders of this group were also interest-

ing. In 6 of the cases, both polymorphonuclear leukocytes and lymphocytes were seen, while in 3 others lymphocytes alone were noted.

TABLE I
Cases Under 1 Year of Age

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver				Gall-bladder		Cause of death
					Portal infiltration	Myelocytes	Polymorpho-nuclears	Connective tissue	Leukocytes	Lymphocytes	
343	1 hr.	M	-	-	1	+	Many	-	o	o	Congenital absence of rectum
344	11 hr.	M	-	-	1	-	Many	-	+	..	Asphyxia
9	N. B.	F	-	-	1	+	Many	-	+	..	Birth trauma
10	N. B.	M	-	-	1	+	Many	-	..	+	Birth trauma
75	N. B.	M	-	-	2	+	Many	-	-	-	Prematurity, birth trauma
40	2 hrs.	M	-	-	2	+	Many	-	..	+	Prematurity
425	N. B.	M	-	-	3	+	Many	-	+	..	Birth trauma
7	3 mos.	M	-	-	1	-	Many	-	o	o	Bronchopneumonia
11	6 mos.	F	-	-	1	-	Few	-	..	+	Meningitis, otitis media
77	5 mos.	F	-	-	1	-	Many	-	-	-	Bronchopneumonia
87	1 mo.	M	-	-	1	-	Many	-	-	-	Inanition
96	6 mos.	M	-	-	1	-	Many	-	+	..	Otitis media, cervical cellulitis
67	4 mos.	M	-	-	1	-	Few	-	o	o	Bronchopneumonia, empyema
66	2 mos.	M	-	-	-	-	Few	-	+	..	Bronchopneumonia
43	1 mo.	M	-	-	3	-	Many	-	+	..	Peritonitis, bronchopneumonia

In all tables ± = minimal infiltration; + = presence of cells indicated; - = absence of cells indicated; o = no observation; 1, 2, 3, and 4 = degree of portal infiltration or cholecystitis.

N.B. = new born; C.P.C. = chronic passive congestion; Tbc. tuberculosis; Carc. = carcinoma.

These cells, however, did not occur in sufficient numbers to indicate an inflammatory process and lymphocytes are known to occur

normally in the gall-bladders of infants. With these findings, it is interesting to speculate as to whether or not many of the minimal cellular reactions, seen in the gall-bladders of adults removed surgically, really represent a pathological process.

In the 1 to 10 age group (Table II) the degree of periportal infiltration was less marked than in any other single group. In 1 case there was no portal infiltration and in 3 others there was only a minimal degree. Only 1 case showed any evidence of cholecystitis. This gall-bladder showed a definite inflammatory change that was unquestionably of clinical significance. The patient died as the

TABLE II
Cases From 1 to 10 Years of Age

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
42	3 yrs.	M	—	—	—	—	—	—	Mastoiditis
329	4	F	—	—	±	Few	—	—	Skull fracture, bronchopneumonia
354	5	M	—	—	±	Few	—	—	Skull fracture
407	3	M	—	—	±	Few	—	—	Diphtheria (laryngeal)
370	3	M	—	—	1	Few	—	—	Appendicitis, peritonitis
65	1	M	—	—	1	Many	—	—	Ulcerative colitis
99	2	F	Tbc.	—	1	Few	—	—	Pulmonary tuberculosis
332	8	M	Torn	—	2	Few	—	—	Ruptured liver
418	8	M	—	—	3	Many	—	3	Skull fracture
100	3	F	—	—	3	Many	—	—	Scarlet fever

result of a skull fracture and no clinical history was available relative to gall-bladder complaints.

In the next 2 groups, namely the 10 to 20 and 20 to 30 age groups (Tables III and IV), the finding of a periportal infiltration in varying degrees was a constant factor, and sometimes it was quite pronounced. In these younger age groups it is interesting to note the high percentage of cholecystitis that was found. Eusterman,²⁶ Snyder²⁷ and others have emphasized the fact that cholecystitis is more frequent in young individuals than is usually recognized. Eusterman reviewed a series of cases seen in the Mayo Clinic and

In the younger age groups there seemed to be some suggestion that in cases of generalized infection the infiltration in the portal spaces was more marked, but in the older groups this association

TABLE VI
Cases From 40 to 50 Years of Age

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
325	42	M	Fatty	—	1	Many	—	0	Skull fracture
382	46	M	—	Thick	1	Few	—	2	Cerebral hemorrhage
435	41	F	Scar	Thick	1	Few	—	2	Valve defect, pneumonia
2	43	M	C. P. C.	—	1	Many	—	—	Skull fracture, meningitis
6	44	M	—	—	1	Few	—	—	Pulmonary tuberculosis
16	47	F	Scar	—	1	Few	—	—	Huntington's chorea
82	46	M	—	—	1	Many	—	—	Bronchopneumonia
89	49	F	—	—	1	Few	—	—	Carcinoma of the ovary
95	49	F	—	—	1	Few	—	—	Pulmonary tuberculosis
1202	40	M	—	—	1	Few	—	2	Ruptured kidney, pneumonia
2	48	F	—	—	1	Few	—	—	Hypertensive kidney
6	40	M	—	—	1	Few	—	—	Suppurative meningitis
313	42	M	Scar	—	2	Many	—	—	Pulmonary tuberculosis
323	45	F	—	Thick	2	Many	—	—	Bronchopneumonia
348	47	M	—	—	2	Few	—	—	Chronic glomerulonephritis
366	42	M	Fatty	—	2	Few	—	1	Chronic alcoholism, pneumonia
372	40	M	Torn	Stones	2	Few	—	2	Ruptured liver
392	48	M	—	—	2	Many	—	—	Pyonephrosis
29	44	M	—	Opaque	2	Few	—	1	Coronary thrombosis
61	49	M	—	—	2	Few	—	—	Skull fracture, meningitis
101	45	F	—	Opaque	2	Few	—	—	Otitis media, meningitis
1385	40	M	Scar	—	2	Many	—	—	Fractured spine
15	42	M	—	—	2	Many	—	1	Pulmonary tuberculosis
17	45	F	C. P. C.	Thick	2	Many	—	2	Bacterial endocarditis
20	44	M	—	—	2	Few	—	1	Fractured spine, myelitis
365	40	M	—	—	3	Many	—	—	Gunshot wound
49	47	M	—	—	4	Many	—	2	Carcinoma of stomach
342	41	M	—	—	4	Few	—	1	Undetermined, poison (?)

was not at all evident. With the idea of discovering whether or not the presence in the gastro-intestinal tract of a chronic ulcerative lesion influenced the degree of infiltration in the liver, those cases showing this type of lesion were tabulated separately (Table XI).

TABLE VII
Cases From 50 to 60 Years of Age

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
327	59	M	—	—	1	Few	—	—	Carcinoma of pharynx
364	51	F	Torn	—	1	Few	—	1	Skull fracture
387	52	M	—	Thick	1	Few	—	1	Hypertensive heart
408	52	M	—	Thick	1	Few	—	2	Hypertensive heart
441	50	M	Scar	Thick	1	Few	—	2	Skull fracture
4	59	F	Scar	—	1	Few	—	—	Cerebral malacia, pneumonia
54	53	M	—	—	1	Few	—	—	Cerebral hemorrhage
73	55	F	Tumor	—	1	Few	—	—	Carcinoma of the breast
368	50	F	Scar	—	2	Few	—	—	Bronchiectasis, pneumonia
404	53	F	—	—	2	Many	—	—	Pulmonary embolus
428	58	M	—	Thick	2	Few	—	Carc.	Bronchiectasis, pneumonia
442	50	M	—	—	2	Few	—	—	Skull fracture
444	50	M	—	—	2	Few	—	—	Skull fracture
28	50	M	Scar	Stones	2	Many	+	—	Skull fracture, endocarditis
31	50	M	—	—	2	Few	—	—	Intestinal obstruction
34	52	F	—	Stones	2	Many	—	—	Hypernephroma, pneumonia
74	51	F	—	Stones	2	Few	—	—	Valve defect, cerebral malacia
88	52	M	—	Thick	2	Few	—	1	Carcinoma of the testicle
93	53	M	—	—	2	Few	—	1	Cyanide poisoning
10	52	F	Scar	Stones	2	Few	—	1	Cerebral malacia
90	50	M	C. P. C.	Opaque	2	Few	—	—	Lobar pneumonia, cerebral malacia
349	55	M	Scar	—	3	Many	+	—	Lobar pneumonia
381	53	M	—	—	3	Many	—	1	Perforated gastric ulcer
394	55	M	—	Thick	3	Few	—	2	Luetic aortitis, pneumonia
47	59	F	Tumor	Thick	3	Few	—	1	Carcinoma of breast, pneumonia
341	50	F	Rough	—	4	Few	+	—	Cirrhosis of the liver
22	50	M	Rough	Opaque	4	Many	+	2	Cirrhosis of the liver
33	52	M	Rough	—	4	Many	+	—	Mastoiditis, meningitis
88	55	M	—	Thick	4	Many	+	1	Hypertension, pneumonia

It seemed reasonable to think that if the infiltration was secondary to infection reaching the liver from the gastro-intestinal tract through the portal system, in the presence of a chronic ulcerative

TABLE VIII

Cases From 60 to 70 Years of Age

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
322	64	M	Scar	Thick	1	Few	-	-	Gangrene of leg, infarcts of lung
328	60	M	-	Opaque	1	Many	-	-	Pulmonary tuberculosis
335	61	M	Scar	Opaque	1	Few	-	-	Skull fracture
410	60	M	-	-	1	Few	-	2	Valve defect
419	60	M	-	Thick	1	Few	-	2	Hypertensive heart, pneumonia
439	67	M	-	-	1	Few	-	-	Carcinoma of prostate, pneumonia
63	67	M	-	-	1	Few	-	-	Lobar pneumonia
64	67	M	-	Opaque	1	Few	-	-	Peritonitis, pneumonia
80	68	M	Scar	Stones	1	many	-	1	Carcinoma of prostate, pyelonephritis
277	60	M	-	-	1	Few	-	-	Carcinoma of bladder, pyelonephritis
330	68	M	Rough	Opaque	2	Many	-	-	Hypertension, uremia
334	69	F	Tumor	Stones	2	Few	-	3	Carcinoma of pancreas
345	68	M	-	Stones	2	Few	-	3	Hypertension, pulmonary infarcts
350	65	M	Scar	-	2	Few	-	1	Cerebral hypertension, pneumonia
363	66	M	-	Stones	2	Few	-	-	Lobar pneumonia
376	69	F	C. P. C.	Stones	2	Many	-	4	Coronary sclerosis
380	66	M	-	-	2	Many	-	0	Carcinoma of esophagus, pneumonia
409	61	M	-	Thick	2	Few	-	2	Hypertension, cerebral malacia
443	63	M	-	Thick	2	Many	-	2	Pernicious anemia
26	64	M	Scar	-	2	Few	-	-	Hypertension, broncho-pneumonia
32	62	F	-	-	2	Few	-	-	Stokes-Adams syndrome
46	68	M	-	Stones	2	Few	-	2	Carcinoma of prostate, pneumonia
50	67	F	Scar	-	2	Many	-	1	Multiple sclerosis, pyonephrosis
51	68	M	-	-	2	Few	-	-	Hypernephroma, erysipelas
57	63	F	-	Stones	2	Few	-	-	Bronchiectasis, pneumonia
83	63	F	Scar	Stones	2	Many	-	1	Valve defect
19	60	F	Scar	-	2	Few	-	-	Diabetes, pericarditis
72	61	M	Rough	Opaque	2	Many	+	2	Valve defect
333	60	M	-	-	3	Many	-	0	Pulmonary tuberculosis
338	61	M	-	Fibrin	3	Few	-	1	Tuberculous peritonitis

TABLE VIII (Continued)

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
357	67 yrs.	F	Tumor	Stones	3	Many	+	Carc.	Carcinoma of the gall-bladder
390	62	M	Tumor	Thick	3	Few	—	—	Carcinoma of the esophagus
424	64	F	—	Stones	3	Many	—	Tumor	Carcinoma of the stomach
438	62	M	Tumor	Thick	3	Few	—	1	Carcinoma of colon, peritonitis
48	65	M	—	Stones	3	Many	—	3	Subdiaphragmatic abscess
314	62	M	Rough	Opaque	4	Few	+	4	Carcinoma of colon
324	60	F	Rough	Opaque	4	Few	+	2	Cirrhosis of liver, valve defect
353	64	F	Rough	Stones	4	Many	+	3	Cirrhosis of liver, peritonitis
371	61	M	Scar	Stones	4	Many	—	1	Bronchopneumonia
78	66	M	Abscess	Stones	4	Many	—	3	Appendicitis, peritonitis

lesion the intestinal filter would be damaged sufficiently to allow more than a normal number of bacteria to reach the liver and this increase would be manifested by an increase in portal space infiltration.

It was found that Grade 1 infiltration occurred in 33.3 per cent of the cases showing chronic gastro-intestinal ulcers, Grade 2 in 37.5 per cent, Grade 3 in 20.8 per cent and Grade 4 in 8.3 per cent. In the entire series, Grade 1 infiltration occurred in 36.7 per cent of the cases, Grade 2 in 40.5 per cent, Grade 3 in 13.6 per cent and Grade 4 in 6.1 per cent. While there is a slight increase in the percentage of the higher grades of infiltration, it is doubtful if the increase is sufficient to be significant in such a small group of cases.

Clinical cholecystitis is generally considered to occur in the ratio of about two females to one male. In this series of autopsy cases, however, the percentage of males showing cholecystitis was 41.9 and the percentage of females 46.2 (Table XII). This ratio is roughly in agreement with Mentzer²¹ who reported 57 per cent of males and 64 per cent females with cholecystitis in a series of 612 autopsies.

TABLE IX

Cases From 70 to 80 Years of Age

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
321	77	F	C. P. C.	—	1	Few	—	—	Bronchiectasis, pneumonia
337	70	M	Scar	—	1	Few	—	—	Pulmonary infarcts, gangrene of leg
358	79	F	Scar	—	1	Few	—	—	Hypertension
377	78	M	—	Opaque	1	Few	—	2	Hypertension
386	73	M	—	Opaque	1	Few	—	—	Bronchopneumonia
397	70	F	Scar	—	1	Few	—	—	Hypertension
407	76	M	Scar	—	1	Few	—	2	Fractured ribs, pneumonia
430	73	M	Scar	—	1	Few	—	—	Hypertension, pneumonia
431	74	M	Scar	—	1	Many	—	—	Fractured pelvis
39	74	M	—	—	1	Few	—	1	Gastric ulcer, pneumonia
44	71	M	—	—	1	Many	—	—	Hypertrophied prostate, pyelonephritis
62	73	F	—	—	1	Few	—	1	Bronchopneumonia
8	77	M	Scar	—	1	Few	—	—	Fractured spine, cerebral hemorrhage
83	71	M	—	Opaque	1	Few	—	1	Lobar pneumonia
315	70	F	Scar	—	2	Few	—	—	First and second degree burns
317	73	M	Scar	—	2	Many	—	2	Pyelonephritis, valve defect
340	77	M	C. P. C.	—	2	Many	—	—	Hypertension, abscesses of kidney
359	75	F	—	—	2	Many	—	—	Fractured femur, gangrenous cystitis
378	72	M	—	—	2	Few	—	0	Hypernephroma, pneumonia
383	73	M	—	—	2	Many	—	—	Skull fracture
398	73	M	C. P. C.	Opaque	2	Few	—	—	Coronary sclerosis
399	71	M	—	Thick	2	Many	—	1	Prostatic abscess, peritonitis
422	76	F	—	—	2	Many	—	1	Carcinoma of stomach, peritonitis
426	77	M	Rough	Stones	2	Few	—	—	Carcinoma of stomach, pneumonia
429	72	M	Rough	—	2	Few	+	0	Cerebral hemorrhage
48	73	M	Scar	—	2	Few	—	—	Bronchopneumonia
92	73	F	—	Opaque	2	Few	—	1	Fractured femur, pneumonia
102	77	F	—	Stones	2	Many	—	1	Diabetes, pneumonia
109	73	M	—	—	2	Few	—	—	Valve defect
85	71	F	—	—	2	Few	—	—	Hemorrhage from gastric ulcer
427	77	F	—	Stones	3	Many	—	2	Perforated gastric ulcer, peritonitis
440	77	F	Rough	—	3	Few	—	1	Valve defect, pneumonia
447	76	M	—	Stones	3	Many	+	—	Lobar pneumonia
5	73	F	Scar	—	3	Few	—	1	Lobar pneumonia
94	74	M	Scar	—	3	Many	—	—	Fractured skull, peritonitis

sies, his higher percentages being due to the fact that he included cholesterosis in his cholecystitis group.

The age distribution in the cases showing cholecystitis is shown in Table XIII. The highest percentage of cholecystitis is seen in the 10 to 20, 20 to 30 and 60-70 age groups. It is doubtful, how-

TABLE X
Cases 80 Years of Age and Over

Number of case	Age	Sex	Gross liver	Gross gall-bladder	Microscopic liver			Microscopic gall-bladder	Cause of death
					Portal infiltration	Polymorpho-nuclears	Connective tissue		
374	85 yrs.	F	Scar	Stones	1	Few	-	1	Hypertensive heart
446	85	F	Scar	-	1	Few	-	-	Gangrenous cystitis, pneumonia
17	84	F	-	Stones	1	Few	-	3	Rheumatic endocarditis, pneumonia
25	87	F	Scar	-	1	Few	-	2	Rheumatic endocarditis, pneumonia
384	93	M	-	-	1	Few	-	-	Coronary sclerosis
351	83	M	-	Stones	2	Few	-	-	Fractured spine
379	81	M	Rough	Stones	2	Few	-	2	Hypertensive kidney, pneumonia
412	83	M	-	-	2	Few	-	-	Carcinoma of stomach, peritonitis
445	81	F	-	-	2	Few	-	-	Fractured femur, pneumonia
12	82	M	-	Opaque	2	Few	-	-	Fractured tibia, pneumonia
69	82	F	-	-	2	Few	-	1	Lobar pneumonia, coronary sclerosis
360	102	M	-	-	2	Few	+	-	Hypertrophied prostate, pneumonia
421	85	M	Scar	-	3	Few	-	-	Hypertrophied prostate
436	89	F	-	Stones	3	Many	-	-	Bronchopneumonia

ever, if the number of cases in several of the decades is sufficient to draw conclusions.

In Table XIV the cases where cholecystitis was present are compared with the cases showing normal gall-bladders with reference to the degree of periportal infiltration. While there is a gradual increase in the percentage of cholecystitis, which roughly parallels

the degree of portal infiltration, many cases with grade 2 and 3 portal infiltration show normal gall-bladders.

In studying the original arrangement of cases by decades, the impression is gained that portal infiltration becomes more severe

TABLE XI

Findings in Cases Showing Chronic Ulcerative Lesions of the Gastro-Intestinal Tract

Number of case	Age	Portal infiltration	Microscopic gall-bladder	Intestinal lesions	Cause of death
	yrs.				
438	62	3	1	Carcinoma of colon	Carcinoma of colon, peritonitis
314	62	4	4	Carcinoma of colon	Carcinoma of colon
49	47	4	2	Carcinoma of stomach	Carcinoma of stomach, pneumonia
424	64	3	—	Carcinoma of stomach	Carcinoma of stomach
422	76	2	1	Carcinoma of stomach	Carcinoma of stomach, peritonitis
426	77	2	—	Carcinoma of stomach	Carcinoma of stomach, pneumonia
412	83	2	—	Carcinoma of stomach	Carcinoma of stomach, peritonitis
389	16	2	—	Tbc. enteritis and colitis	Pulmonary tuberculosis
70	29	1	—	Tbc. enteritis and colitis	Pulmonary tuberculosis
91	21	1	—	Tuberculous colitis	Pulmonary tuberculosis
95	49	1	—	Tbc. enteritis and colitis	Pulmonary tuberculosis
15	37	1	—	Tbc. enteritis and colitis	Pulmonary tuberculosis
92	73	2	1	Ulcerative colitis	Fractured femur, pneumonia
29	44	2	1	Ulcerative colitis	Hypertension, coronary thrombosis
381	53	3	1	Gastric ulcer (ruptured)	Perforated gastric ulcer, peritonitis
401	19	1	1	Gastric ulcer (ruptured)	Abscesses of liver, peritonitis
330	68	2	—	Gastric ulcer	Hypertension, uremia
337	70	1	—	Gastric ulcer	Hypertension, pneumonia
427	77	3	2	Gastric ulcer	Perforated gastric ulcer, peritonitis
39	74	1	1	Gastric ulcer	Pneumonia
85	71	2	—	Gastric ulcer	Hemorrhage from gastric ulcer
440	77	3	1	Duodenal ulcer	Valve defects, pneumonia
374	85	1	1	Duodenal ulcer	Hypertension
379	81	2	2	Duodenal ulcer	Hypertension, uremia, pneumonia

as age increases. In Table XV the percentage of infiltration for each age group and each degree of infiltration are charted. It is evident from these figures, however, that the increase in age is not paralleled by an increase in infiltration in any of the four grades.

TABLE XII
Sex Distribution

Age	Number of cases		Cholecystitis		% Cholecystitis	
	Male	Female	Male	Female	Male	Female
yrs.						
20-30.....	7	10	3	7		
30-40.....	8	8	4	2		
40-50.....	20	8	9	2		
50-60.....	19	10	9	3		
60-70.....	29	11	15	6		
70-80.....	22	13	6	7		
80+.....	7	7	1	4		
Totals.....	112	67	47	31	41.9	46.2
Under 1	12	3	0	0		
1-10.....	7	3	0	1		
10-20.....	5	3	3	1		
Totals.....	24	9	3	2	12.5	22.2

TABLE XIII
Age Distribution

Age	Number of cases	Cholecystitis	% Cholecystitis
yrs.			
Under 1	15	0	0
1-10.....	10	1	10.0
10-20.....	8	4	50.0
20-30.....	17	10	58.8
30-40.....	16	6	37.5
40-50.....	28	11	39.2
50-60.....	29	12	41.3
60-70.....	40	21	52.5
70-80.....	35	13	37.1
80+.....	14	5	35.7
Totals	212	83	39.1

TABLE XIV
Comparison of Degree of Portal Infiltration

Liver infiltration	Cholecystitis		No cholecystitis	
	No. cases	Per cent	No. cases	Per cent
None	0	0	2	100
±.....	0	0	4	100
1.....	23	29.5	55	70.5
2.....	35	40.6	51	59.4
3.....	15	51.7	14	49.3
4.....	11	84.6	2	15.4

TABLE XV

Age Distribution of Portal Infiltration

Portal infiltration	Age 1-10 yrs.		Age 10-20 yrs.		Age 20-30 yrs.		Age 30-40 yrs.		Age 40-50 yrs.		Age 50-60 yrs.		Age 60-70 yrs.		Age 70-80 yrs.		Age 80 + yrs.	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Neg.	1	10.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
±	3	30.0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1 +	3	30.0	4	50.0	6	35.2	6	37.5	12	42.8	8	27.5	10	25.0	14	40.0	5	35.7
2 +	1	10.0	3	37.5	5	29.4	8	50.0	13	46.4	13	44.8	18	45.0	16	45.7	7	50.0
3 +	2	20.0	1	12.5	5	29.4	0	0	1	3.5	4	13.7	7	17.5	5	14.2	2	14.2
4 +	0	0	0	0	1	5.8	1	6.3	2	7.1	4	13.7	5	12.5	0	0	0	0

DISCUSSION

Study of this series of cases shows conclusively that an infiltration of lymphocytes and polymorphonuclear leukocytes in the portal connective tissue is almost a constant finding in livers from autopsy cases. In this series of 212 cases the portal spaces were free from inflammatory cells in only 2 instances. In 3 other cases the reaction was practically negligible. From these minimal changes the lesion ranged in intensity to the Grade 4 reaction shown in Figure 8. A marked variation in degree was noted in different areas of the same section and only by repeated study of the sections was it possible to arrive at a fair estimate of the intensity of the reactions. As mentioned above, the liver edge showed more intense changes in many cases, both from the standpoint of inflammatory cells and increased connective tissue, than areas well away from the capsule and edge, so that it is justifiable to say that the liver edge is not a reliable source of tissue from which to judge the histology of the whole organ. The published descriptions of the hepatitis accompanying cholecystitis are on the whole rather vague, Tietze and Winkler's²⁰ description being the only exception, but apparently the basis for all of the accepted conceptions of hepatitis is the presence of inflammatory cells in the portal connective tissue. With the exception of Mentzer²¹ and Aiello²³ these studies have been made on surgical material in known cases of cholecystitis, and because in these cases this lesion was found it was reasoned that the lesion must be in some way associated with the etiology of cholecystitis, in spite of the fact that it is seen almost constantly by pathologists who examine routine sections of autopsy material. Mentzer²¹ came to the conclusion that "60 to 70 per cent of all livers show evidence of pathological changes at postmortem examination, regardless of the presence or absence of gall-bladder disease." He goes on to say, however, that in the case of minor gross lesions of the gall-bladder, such as diverticula and cholesterosis, the percentage of liver involvement is about 60 "as compared with definitely inflammatory diseases, such as gall stones," where 97 per cent of the livers showed hepatitis. In this series of cases Mentzer's conclusions were not borne out.

This study revealed no definite explanation for the almost constant finding of inflammatory cells in the liver. Both Graham⁵

and Judd¹⁶ admitted finding hepatitis identical with that seen in conjunction with cholecystitis in cases showing no evidence of gall-bladder infection, and one is forced to favor Martin's²² idea that the inflammation is secondary to the lodgement of bacteria and toxic substances carried to the liver by the portal blood. This conception is given some support by the work of Andrews and Hrdina^{28, 29} who cultured *B. Welchii* from the livers of normal dogs and showed that after the ligation of the cystic and common bile ducts in dogs, a cholecystitis was produced that first involved the portion of the gall-bladder adjacent to the liver. Arnold³⁰ found that in dogs with a loop of small intestine experimentally isolated and stimulated by the presence of food in a medium with a reaction of pH 8 the lymph from the thoracic duct contained 500 to 1000 bacteria per cc. All of these facts seem to favor the intestine as the possible source and the portal blood as the conducting medium for the infection of the liver. The suggestion that the liver may obtain bacteria, under both normal and pathological conditions, is by no means new. Adami,^{31, 32} in 1898 studied autopsy specimens of liver and was able to show numerous microorganisms in cases of cirrhosis. He thought these bacteria were *B. coli*. He was able also to demonstrate bacterial shadows in cases showing no liver pathology, and he was of the opinion that they represented bacteria in the process of destruction by the endothelium of the liver.

As mentioned above, none of the cases under consideration here were cases of clinical cholecystitis and until such a series is included in this group no definite conclusions can be drawn concerning the degree of liver inflammation in clinical cholecystitis. Most workers agree, however, that even in the presence of severe gall-bladder infection, the liver lesions were sometimes minimal, and certainly the Grades 3 and 4 cholecystitis seen in this series are comparable in degree to many well established clinical cases of chronic cholecystitis. There seems to be, however, a definite difference between the cholecystitis found at autopsy and the clinical disease. This is perhaps best shown in the matter of age and sex distribution. The autopsy lesion certainly occurs more frequently in the younger age groups than does clinical cholecystitis and, whereas in postmortem material the disease occurs almost equally in the two sexes, clinically the female is affected at least twice as often as the male. In the case of the lesser degrees of infection in the autopsy cases some

doubt may be thrown on their interpretation as true or at least significant infections, although in most instances they are as marked as those seen in certain specimens removed surgically.

The occurrence of increased connective tissue in the portal spaces, as demonstrated in this series, was too infrequent to be of any practical importance in the consideration of the etiology of cirrhosis. In 17 cases such an increase was noted and in 5 of these cases the lesion was well advanced and presented the picture of typical portal cirrhosis. In the other 12 cases the increase in connective tissue was recognized only on microscopic examination.

CONCLUSIONS

1. Infiltration of the portal spaces by lymphocytes and polymorphonuclear leukocytes is seen almost constantly in the liver at autopsy.

2. This portal infiltration seems to have no particular relation to generalized infections or to any specific type of disease.

3. The portal infiltration apparently bears no relation to the non-clinical cholecystitis frequently found in routine postmortem examinations.

4. The cholecystitis found in routine postmortem examinations is seen less frequently in the female than clinical cholecystitis and it is more frequent in the younger age groups than clinical cholecystitis.

5. In this autopsy series cirrhosis occurs too rarely to justify the conclusion that the hepatitis described has any relation to the etiology of cirrhosis.

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DESCRIPTION OF PLATES

PLATE 70

Sections of the wall of the gall-bladder showing the respective degrees of cholecystitis. Figures 1, 2, 3, and 4 show respectively the corresponding grades of the inflammation.



1



2



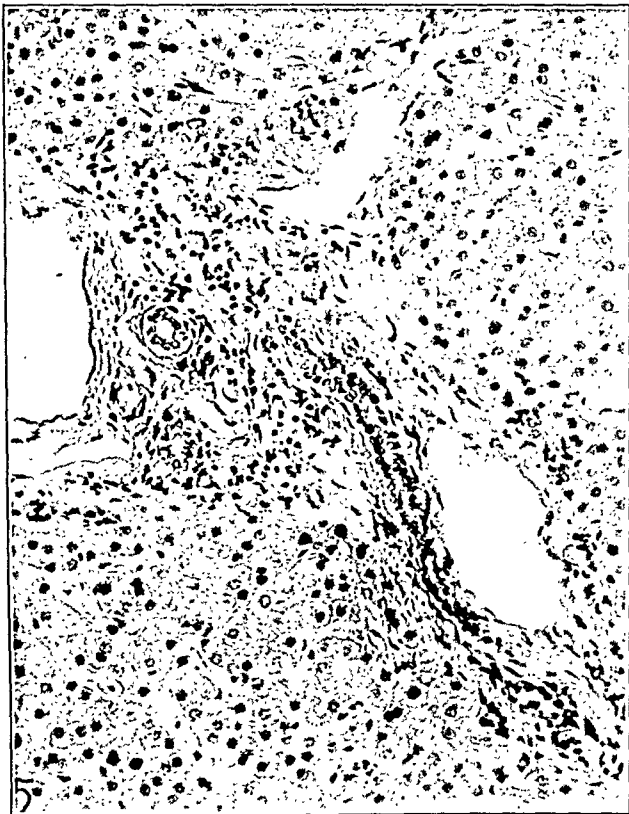
3



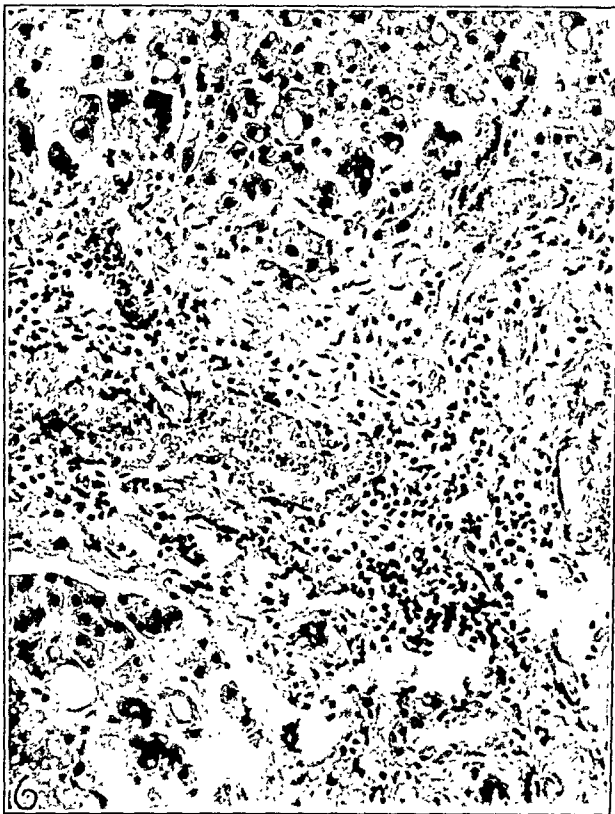
4

PLATE 71

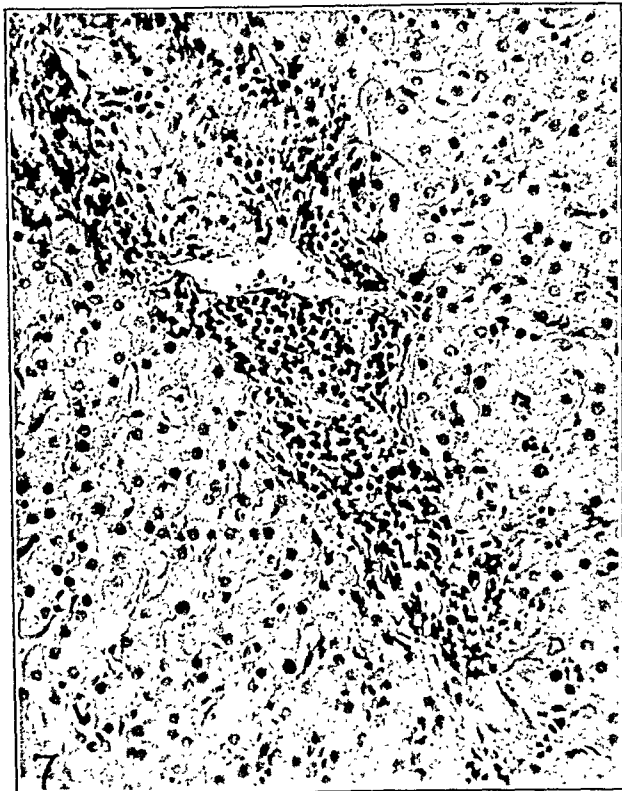
Sections of the liver showing the degrees of portal infiltration. Figures 5, 6, 7 and 8 correspond respectively to Grades 1, 2, 3, and 4 of portal infiltration.



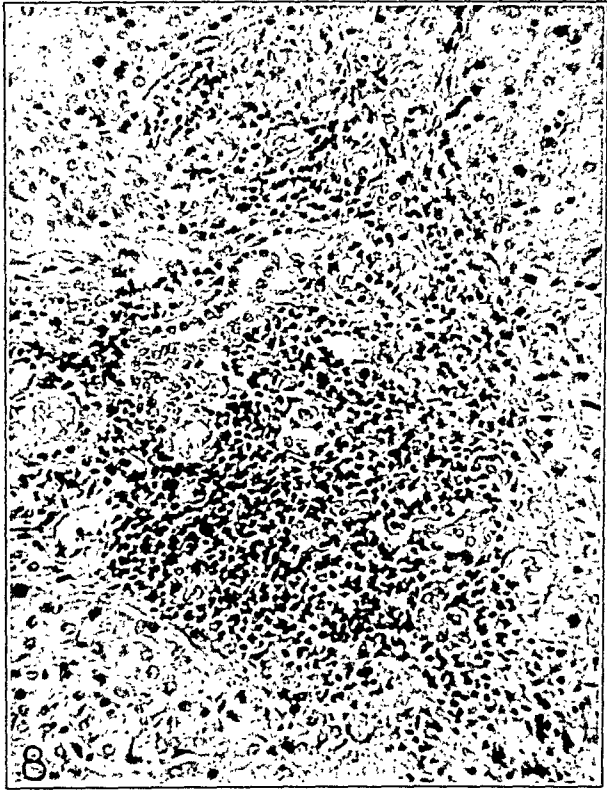
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6



7



8

CALCIFIED EPITHELIOMA OF THE SKIN*

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INTRODUCTION

The so-called "calcified or calcifying epithelioma" of the skin is rarely mentioned in the English literature. I have been able to find only 1 case diagnosed as calcified epithelioma of the skin, reported by Nicholson in 1917, and 2 similar cases described as calcified adenoma of the skin, 1 reported by Eve and the other by Hutchinson. Even so comprehensive a work as Ewing's *Neoplastic Diseases* makes no mention of them. On the other hand, these tumors are not infrequently reported and discussed by German and French authors. In the Department of Pathology of Peiping Union Medical College 10 cases have come to our notice in the past 15 years, during which time more than 22,000 surgical specimens have been examined. In comparison with the ordinary malignant squamous cell epitheliomas and the epidermoid (sebaceous) cysts of the skin the infrequency of this type of tumor is remarkable and sufficient to warrant a report of this kind, especially in view of the scantiness of the English literature on the subject.

The detailed descriptions of our 10 cases are given in the case reports to follow. The important features that are presented in Table I can be summarized as follows: The cases are equally divided between two sexes, with an age incidence ranging from 13 to 29 years. One-half of the cases occur before the age of 20 years. In 7 of the cases the tumors are found on the head (5 on face, 2 on neck) and in 3 cases on the arm. The tumors are hard or firm, 0.5 to 9.5 cm. in diameter, sharply encapsulated, subcutaneously situated, freely movable over the deeper tissues but usually adherent to the overlying intact skin. Their cut surfaces show a characteristically sandy or gritty appearance.

Microscopically the parenchyma consists chiefly of degenerated epithelial cells, but isolated areas of living cells are usually to be found. These latter cells are small, oval, deeply staining and closely

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packed without distinct cell borders, but here and there they are squamous in shape with cornification and pearl formation. Mitotic figures are as a rule difficult to find. The degenerated cells stain a deep pink color in hematoxylin eosin sections and their outlines are generally well preserved. The stroma is composed of strands or a network of hyalinized or cellular fibrous tissue that varies in amount in different places and that is continuous with the fibrous capsule. Cholesterin crystals and other fatty substances may be found in the degenerated epithelium and in the stroma. Calcification is constantly present in the degenerated epithelium, either as fine dusting or as irregular lumps, and in the stroma either as incrustation of the fibrous fibers or as irregular granules. Bone tissue is frequently laid down from the periphery inward to replace the calcified epithelial masses. Surrounding the cholesterin crystals and calcified areas foreign body giant cells are frequently seen.

REVIEW OF LITERATURE

Nomenclature: The names by which these tumors are designated by different authors who have reported them are not uniform. "Calcified epithelioma" ("verkalktes Epitheliom," "épithéliome calcifié") is by far the most popular name employed. They have been variously regarded as endotheliomas (Perthes); sebaceous gland adenomas (Eve, Barlow, Hutchinson); atheromas or epidermoids (Joannevics, Sternberg, Krüger (quoted from Gans), Virchow); carcinomas (Strassberg, Frey, Förster, von Noorden, Denecke, Bilke (quoted from Gans)); specially modified dermoids (Souligoux and Pilliét); or even as papillomas in dermoid cysts (Linser). That these authors are speaking about the same sort of tumor can be determined by reading their descriptions and examining their plates or illustrations. Perthes thinks that his "calcified endothelioma" is not different histologically from the "calcified epithelioma" of Malherbe and Chenantais, who published the first report in 1880, and attributes the discrepancy in name merely to difference in interpretation. Reverdin mentions the fact that 2 of his cases look like endotheliomas. Firket thinks that calcified epithelioma and endothelioma belong to the same category. Barlow thinks the "cancroids" of Förster, the "epitheliomas" of Chenantais and von Noorden, and the "sebaceous adenomas" of Eve and Hutchinson are the same thing and

TABLE I

Case No.	Sex	Age	Location	Duration	Diameter	Consistence	Free-movability	Adherence to overlying skin	Fibrous capsule	Epithelial parenchyma						Stroma			Fat				Cholesterol crystals		Calcification		Ossification	Cystic spaces
										Small, deeply-staining, without distinct cell borders	Squamous, with distinct cell borders	Mitoses	Epithelial pearls	Corrification	Necrotic cells	Hyalinized or cellular	Foreign body giant cells	Lymphocytic infiltration	Neutral	Fatty acid	Neutral	Fatty acid	In necrotic thelium	In stroma	In parenchyma	In stroma		
1	M	26 yrs.	Eyelid	1 yrs.	1.5 cm.	Hard	?	?	+	+	-	-	+	+	+	H	+	+	o	o	o	o	+	+	+	+	+	+
2	M	29	Left temporal	1½	1.2	Hard	+	+	+	+	-	-	+	+	+	H	+	+	o	o	o	o	+	+	+	+	+	+
3	M	26	Right temporal	1	3.0	Hard	+	+	?	+	-	-	+	+	+	C	-	-	o	o	o	o	+	+	+	+	+	+
4	M	20	Neck and face	?	3.0	Hard	+	+	+	-	-	-	-	-	+	H	+	+	-	+	+	+	+	+	+	+	+	+
5	M	22	Right arm	2	9.5	Hard	+	+	+	+	+	-	+	+	+	H	+	-	-	+	+	+	+	+	+	+	+	+
6	F	13	Forehead	3	2.2	Firm	+	+	+	+	+	-	-	+	+	HC	+	-	-	+	+	+	+	+	+	+	+	+
7	F	23	Left arm	½	3.0	Firm	+	+	+	+	+	+	+	+	+	HC	+	+	-	+	+	+	+	+	+	+	+	+
8	F	18	Right temporal	4	2.5	Hard	+	-	+	-	-	:	+	+	+	H	+	+	o	o	o	o	+	+	+	+	+	+
9	F	16	Left arm	½	0.5	Firm	+	+	+	+	+	-	+	+	+	C	+	-	o	o	o	o	+	+	+	+	+	+
10	F	20	Left mastoid region	8-9	1.2	Firm	+	-	+	-	-	:	-	-	+	C	+	+	o	o	o	o	+	+	+	+	+	+

o = No tissue available for fat reaction.

H = Hyalinized.

C = Cellular.

prefers the last-mentioned name. Malherbe and Chenantais call these tumors "calcified epitheliomas of the sebaceous gland," and Saltykow uses the name "calcified epithelioma" for a type of tumor that he thinks arises from sebaceous gland anlage. Frey designates his tumor "psammocarcinoma of the skin." Gans distinguishes such tumors from true squamous cell epitheliomas with calcification or ossification by the presence of a capsule in the former and its absence in the latter. He also thinks that a so-called calcified epithelioma, before it begins to calcify, is histologically identical with a basal cell carcinoma, but this diagnosis is excluded by the sharp encapsulation of the tumor. Saltykow also shares this view. Strassberg and Dubreuilh and Cazenave are of the opinion that "calcified epithelioma" is not a good name because the calcification, being observed also in the common squamous epitheliomas of the skin, is not a characteristic process; it is the necrosis alone that is the essential feature. Bilke also thinks of calcification as a secondary process. Since the name "calcified epithelioma" has already been universally accepted to denote a clinically well defined type of tumors, it may be conserved (Dubreuilh and Cazenave).

Histogenesis: These tumors are by the consensus of opinion traced to an epithelial genesis. They are believed by different authors to arise from preformed glands, dystopic deep-lying epithelial cells and misplaced anlage material (Kaufmann). Saltykow, Firket, Murakami and Lücke favor the origin from epithelial cell rests. Reverdin thinks that the ectodermal inclusion may be of either embryonal or traumatic nature. Jadassohn (referred to by Gans), Dössekker, Bilke, and in certain respects also Firket, Murakami and Saltykow, believe in a sebaceous gland anlage as the source of these growths. Frey considers a kind of embryonic rest of primitive sebaceous gland and primitive squamous epithelium, with the double potentiality of forming fat cells and cornified epithelium in an imperfect and disturbed fashion. This forms a mixed product of incompletely differentiated fat and epithelial cells, which in time are overtaken by calcification. Sternberg, Krüger, Joannevics and Virchow take these tumors to be of the same origin as the epidermoids (sebaceous cysts), but Gans and Bilke are opposed to this view because these tumors, besides the difference in anatomical structures, occur too rarely, as compared with the exceeding frequency of the epidermoids (sebaceous cysts). Jadassohn thinks they are closely related to nevi.

Table 11

Country	Year	Value
Algeria	1962	1,000
Angola	1962	1,000
Argentina	1962	1,000
Australia	1962	1,000
Austria	1962	1,000
Belgium	1962	1,000
Brazil	1962	1,000
Canada	1962	1,000
Chile	1962	1,000
Colombia	1962	1,000
Cuba	1962	1,000
Czechoslovakia	1962	1,000
Denmark	1962	1,000
Egypt	1962	1,000
France	1962	1,000
Germany	1962	1,000
Greece	1962	1,000
India	1962	1,000
Indonesia	1962	1,000
Italy	1962	1,000
Japan	1962	1,000
South Korea	1962	1,000
Malaysia	1962	1,000
Mexico	1962	1,000
Netherlands	1962	1,000
New Zealand	1962	1,000
Norway	1962	1,000
Philippines	1962	1,000
Poland	1962	1,000
Portugal	1962	1,000
Romania	1962	1,000
Saudi Arabia	1962	1,000
Spain	1962	1,000
Sweden	1962	1,000
Switzerland	1962	1,000
Taiwan	1962	1,000
Thailand	1962	1,000
Turkey	1962	1,000
U.S.S.R.	1962	1,000
Uganda	1962	1,000
United Kingdom	1962	1,000
United States	1962	1,000
Yugoslavia	1962	1,000
Total	1962	1,000

Bilke believes they have a marked similarity to cholesteatomas that arise from epidermal inclusions in the central nervous system, and his belief was strengthened when he found clearly lamellated cornified masses in such tumors (Gromiko). Souligoux and Pilliét regard them as related to dermoid cysts because both occur more frequently in young people, and Linser goes so far as to consider a calcified epithelioma as nothing more than a papilloma growing in a closed cavity of a dermoid cyst and filling up the entire cavity and adhering to its walls. Gans thinks that those tumors whose glandular origin is evident are best considered as adenomas or nevi, while a classification for others whose origin is doubtful should be postponed until the studies of the earlier stages of the tumor can be made.

Analysis of Reported Cases as to Body Regions, Sex, and Age: From the literature available to me, 116 reported cases have been collected and are presented in the following table (Table II).

TABLE III

Head and neck	Eyebrow, eyelid or orbit	12 cases
	Neck	9 "
	Forehead	4 "
	Cheek	3 "
	Lower jaw	2 "
	Face	2 "
	Parotid region	2 "
	Ear lobe	2 "
	Temporal region'	1 case
	Occipital region	1 "
	Mastoid region	1 "
	Lower lip	1 "
	Scalp	1 "
	Exact location not specified	16 cases
	Total	57 cases

[illegible][illegible]

The tumors reported in literature are most frequently located on the head and neck, as shown in Table III. Although such tumors usually occur singly, two or more similar growths are occasionally found in different regions of the same individual (Perthes).

Of the 116 reported cases only 35 are found in which the sex of the patient is mentioned. Of these, 23 cases occurred in the female and 12 in the male. Malherbe has the impression that the female sex is predisposed.

There are 31 cases in which the age of the patient is known and they are distributed as follows: 14 below 20 years of age, 8 between 21 and 50, and 9 after 51. Of the 14 cases occurring before 20, 7 are

before 10, and 3 of these 7 before 16 months. The youngest case, reported by Firket, was an infant 2 months old. The view that these tumors are most frequently encountered in youth is shared by Malherbe, Firket, Souligoux and Pilliét, and Reverdin. On the other hand, Kaufmann and Perthes are of the opinion that they occur at any age without special predilection.

Predisposing Causes: The direct cause of these tumors is unknown. Malherbe has three times observed trauma as the exciting cause. In one case the tumor arose after the sting of a wasp.

Description of the Tumor: The calcified epitheliomas constitute a special tumor group by their structure and location. Their appearance is so unique that, if one has not known of such a tumor as calcified epithelioma, the diagnosis is difficult (Saltykow). They form the immense majority of the bony tumors of the skin (Malherbe). They are reported as lying in the cutis, subcutis, or partly in the cutis and partly in the subcutis. They are slow-growing, rounded, stone-hard, cartilaginous or soft, freely movable, sharply encapsulated, at times cystic, and usually have a gritty cut surface. They are usually small, described as hazel-nut or walnut-sized growths. The smallest reported is the case of Sehrt, where the tumor measured only 4 mm. in diameter, while both Murakami and Gromiko have described tumors as large as the head of a newborn. The tumor consists of a connective tissue capsule and stroma with epithelial cells that are in the form of nests, lobulated masses, or in alveolar arrangement.

(a) *The Epithelial Cells:* The epithelial cells are described as typical squamous cells with keratohyalinization and pearl formation (Saltykow, Firket, Kaufmann, Sehrt, Gromiko). According to Eve, however, they are small, round, with deeply staining nuclei, closely packed together with no intercellular substance. Gans finds that before calcification they appear like the cells of a basal cell carcinoma, and he distinguishes in the cell masses an outer and an inner layer of cells, those of the outer layer being more cubical and those of the inner layer more rounded.

The characteristic tendency of the epithelial cells to undergo necrosis has been described by many authors. The necrosis begins usually in the centers of the masses and spreads toward the periphery. The original outline and structural details of the necrotic cells are generally well preserved. In the necrotic epithelial masses

one finds cholesterin crystals, fatty acids and calcium deposits. The latter are either in the form of deeply stained masses or concentric layers, or merely as a fine dusting of the cell cytoplasm.

Cornification of epithelium is, according to Gromiko, a typical feature of this tumor. The cornified epithelium of the calcified epithelioma is distinguished by Thorn from the epithelial pearls of the squamous cell epithelioma by the lack of any definite structure in the former and the presence of nucleated layers of corneum in the latter. Murakami could not find this difference, while Gromiko observed some cornified pearls of the calcified epithelioma quite similar to, but others (being much larger and irregular) different from, those of the squamous cell epithelioma.

(b) *The Connective Tissue Stroma:* The connective tissue stroma is described typically as fibrillar or acellular. It extends from the capsule into the interior of the tumor in the form of septa, strands, or a sort of network, in the meshes of which lie the epithelial masses. The connective tissue fibers may also undergo necrosis and those fibers adjacent to the calcified epithelial masses may also become incrustated with calcium. Cholesterin crystals are found in the degenerated stroma. The stroma frequently shows edematous and hyaline changes, and rarely mucinous and fibrosarcomatous degeneration (Léjard, quoted from Henzi). Foreign body giant cells are usually found around the calcified masses and cholesterin crystals.

(c) *Cystic Spaces in the Tumor:* Cystic spaces or cavities are generally found in the tumor as a result of degeneration or liquefaction of epithelial cells (Kaufmann); edema of the connective tissue (Murakami); shrinkage of volume consequent upon cornification, calcification, and disappearance of tissue fluid (Gans); or contraction following fixation (von Noorden, Gromiko).

(d) *The Peripheral Parts of the Tumor:* The peripheral parts or the immediate vicinity of the tumor, according to Gans and others, show no inflammatory reaction. Such a reaction, however, has been described by Henzi.

(e) *The Skin Overlying the Tumor:* Aside from flattening and stretching consequent upon the expansile growth of the tumor, the overlying skin is not ulcerated or invaded even when the tumor may reach a great size (Gromiko).

Necrosis and Calcification: The great tendency of these tumors to necrosis is ascribed by some to their poor blood supply (Nicholson,

Gromiko, Hutchinson, Sehrt), and by others to the low vitality of their cells (Gromiko). It is generally agreed that calcification follows necrosis. There is reason to believe that, in some cases, calcification is preceded by deposition in the necrotic tissue of fatty acids, which form insoluble soaps with the calcium of the blood or tissue fluid.

Ossification: Ossification is looked upon as a natural sequela of necrosis (Saltykow) and as imparting no additional distinctive feature to the tumor (Malherbe). Wilkens, 1858 and Denecke, 1893 are the earliest authors to describe bone formation in calcified epitheliomas, then called "carcinomas." The bone formation is described as perfectly well formed with lacunae, canaliculi, Haversian systems and marrow cavities. Many authors report bone occurring along the edges of the calcified epithelial masses and in the stroma. The bone formation is preceded by the formation of a granulation tissue that contains embryonic fibroblasts with osteoblastic function (Nicholson). Bone forms at the site of the calcified deposits, which are first dissolved and removed by the giant cells (Strassberg, Saltykow, Denecke, Walkoff, Henzi). Actual marrow elements, *e.g.* eosinophilic and neutrophilic myelocytes and leukocytes (Sehrt); megakaryocytes (Nicholson); nucleated red cells (M. B. Schmidt); and fat cells (Saltykow), have been found in the stroma and in the marrow cavities.

Clinical Behavior: Calcified epitheliomas are generally benign, non-destructive and non-metastasizing. Malherbe says this tumor is almost always benign and he did not know of more than 2 cases with recurrence after operative removal. Reverdin found only 2 recurrent cases out of 53 published observations. Gromiko reports a case with malignant degeneration in a workman 32 years of age. The first tumor, situated on the upper third of the right forearm, was a small, slow-growing, rounded mass that was removed 2 years after it had been noticed. Cut surface of this tumor showed small grains like sand, but no histological examination was made. Nine years after the operation a new tumor appeared on the inner side of the right elbow, growing steadily from a small nodule to the size of the head of a newborn. No history of trauma was given. The consistence of the tumor was hard, and the capsule adherent to deeper tissues. The clinical diagnosis was "sarcoma of right cubital region." The pathological diagnosis was "calcified epithelioma."

Ossification was present. Five months and 8 days after the second operation the patient again noticed a tumor, appearing at the site of the operative scar, that grew rapidly and caused much pain. Pain was also felt in the fourth and fifth fingers of the right hand. The tumor was not movable and amputation of the humerus was performed. The diagnosis was: "calcified epithelioma of skin with malignant degeneration and recurrence." This last tumor showed calcification and ossification in small amounts. Both the second and the third tumors were hemorrhagic, especially the latter. Inside the blood vessels of the stroma and the capsule of both tumors there were found tumor cells that also underwent massive cornification in the same manner as the cells of the tumor parenchyma. Mitotic figures were found in the epithelial cells of both tumors. Gromiko explains the recurrence by the probable presence of tumor cells inside blood vessels in the close neighborhood of the tumor, which were not removed with the tumor mass during operation. According to him, Thorn has also found the tumor cells inside the blood vessels and Denecke has found them inside the lymphatics in the stroma of the tumor.

CASE REPORTS

CASE 1: S-10,122. Specimen sent in from Good Will Hospital, Nanhsuchow, Anhwei, on Dec. 6, 1926. Chinese male, aged 26 years. Tumor of eyelid of 1 year's duration.

Pathological Report: Specimen consists of a piece of tissue measuring about 1.5 cm. in diameter. Consistence hard. On cut section the tissue is grayish white and whitish opaque in color. (No gross specimen has been left; this description is from the routine record.)

Microscopic Examination: There is a fibrous capsule from which trabeculae of hyalinized stroma ramify into the interior of the mass. In between the hyalinized stroma lie masses of epithelial cells that are totally necrotic with nuclei and cytoplasm staining alike with eosin, but whose structural details are remarkably well preserved. Pearl formation and cornification are frequent in these masses. In places the central parts of these dead epithelial masses do not take any stain and are in the form of a very pale granular débris. Here and there among the dead masses one finds, however, isolated islands of living epithelial cells whose nuclei are small, oval or spherical, dark blue-staining, and closely packed in the form of a syncy-

tium without visible cell borders. Transitions, either abrupt or gradual, from these living epithelial cells to the dead cells, are readily found. Numerous giant cells are found at the edges of the necrotic epithelial masses; some are also found free in the stroma tissue. Calcification is seen in the epithelial masses in the form of small dense masses or as irregular, scattered areas. Calcification of the stroma is in the form of incrustation of the connective tissue fibrils. The stroma is infiltrated with small lymphoid cells, especially near the periphery. Many irregular, empty or cystic spaces are found in the tumor.

CASE 2: O. P. D. No. 126348, S-12,408. C. S. J., a Chinese male clerk, aged 39 years, came to Peiping Union Medical College on April 3, 1928, with the complaint of a swelling in the left temporal scalp for $1\frac{1}{2}$ years. It had begun without known cause 18 months before as a small, hard, slipping mass which grew bigger gradually. It had never been painful or tender. Upon examination the swelling was found to be a very hard, non-tender, flattened and freely movable tumor mass 1.5 by 1.5 cm. in diameter. It seemed to be attached to the overlying skin.

Pathological Report: Specimen consists of a nodular mass measuring 1.2 by 0.5 by 0.3 cm. Its external surface is grayish pink in color and in places shows light yellow, elevated nodules. Consistence hard. On section the cut surface is grayish pink with light yellow areas.

Microscopic Examination: The tumor is surrounded by a thin fibrous capsule and consists of a network of ramifying bands of fibrous tissue stroma, in the meshes of which lie necrotic epithelial masses. Although totally necrotic, as judged from the staining qualities, these cells preserve their outline and structural details strikingly well. The cells are small and of squamous type with rather extensive cornification and formation of pearls. Only one of these necrotic masses has a narrow strip of living cells attached to its edge. These living cells are small, oval, closely packed, with deeply staining nuclei without distinct cell borders, and are sharply demarcated from the stroma. These cells resemble the basal cells of the epidermis. Calcification of the necrotic epithelial masses and cornified pearls is extensive and occurs as deep blue areas with stratification, as irregular masses or coarse granules, or as very fine dust particles in the cytoplasm of the dead epithelial cells, whose outlines are still preserved. Calcium granules are also found in the connective tissue fibers that may become themselves so heavily incrustated

with calcium that they appear as coarse, blue, wavy lines. Cholesterol crystals are occasionally found in the necrotic cell masses and frequently found in the degenerated stroma which also shows edema and hyalinization. Giant cells are found at the edges of the necrotic and calcified epithelial masses and also scattered in the stroma. The stroma of the peripheral portion of the tumor is more vascular and more cellular, and infiltrated with more lymphocytes and wandering cells than that of the central portion. Cystic spaces are present apparently as a result of necrosis and degeneration.

CASE 3: O. P. D. No. 129519, S-12,641. P. Y. M., a Chinese policeman, aged 26 years, came to Peiping Union Medical College on May 17, 1928, with the complaint of a painless swelling in the right temporal region for 1 year. Upon examination a tumor of the size of a small walnut was found; it was adherent to the skin but free from the bone of the skull. It was hard or cartilaginous, but soft in places. The diagnosis of sebaceous cyst was made and the tumor excised.

Pathological Report: Specimen consists of several irregular pieces of tissue measuring 0.2 to 1 cm. in diameter. Their external surfaces are rough, and are grayish pink and light yellow in color. The consistence is firm. On section the cut surfaces are grayish white in color and show light yellow, chalky, calcified areas.

Microscopic Examination: The growth consists of ramifying strands of fibrous tissue in the meshes of which lie necrotic masses of epithelial cells of squamous type with rather extensive cornification and pearl formation in their central parts. Although the epithelial cells are necrotic and their nuclei fail to stain, their outline and structural details are still preserved. Only in two or three places are small masses of living epithelial cells found. These are small, oval, with deeply staining nuclei, closely packed and without clear cell borders. They resemble closely the cells of the basal layer of the epidermis. Calcium deposits, often in the form of large and irregular masses, are found in the necrotic epithelial masses. All the cornified pearls are necrotic and without nuclei. Many of them are hyalinized, others are either completely or partially calcified. Irregular masses and coarse granules of calcium are found in the otherwise unchanged stroma and there is also incrustation of the connective tissue fibers with calcium. No giant cells are found. Cellular infiltration is absent in the peripheral as well as in the central region. Cystic spaces are occasionally found.

CASE 4: O. P. D. No. 134730, S-13,013. H. F. Y., a Chinese shoemaker, aged 20 years, came in on July 27, 1928 with the complaint of a rectal abscess. He was accidentally found to have unusual tumor nodules in the posterior cervical region and anterior to the left ear. No history was obtainable about these nodules. They were flattened, very hard or cartilaginous in consistence, freely movable but attached to the overlying skin. One such nodule was excised for biopsy.

Pathological Report: Specimen consists of a hard nodule measuring 3 by 1.5 by 1 cm., well encapsulated and covered in places with subcutaneous fat. On section the cut surface is grayish white in color, showing ramifying strands of bone-like tissue.

Microscopic Examination: The microscopic picture is in many respects identical with that of Case 5 (see below) which was better preserved and therefore will be more fully described. The tumor is encapsulated with fibrous tissue and consists of ramifying strands of hyalinized, fibrillar connective tissue stroma, in the meshes of which lie epithelial cell masses. There are no living, unaltered epithelial cells left. All the epithelial masses are totally necrotic, but their outlines and structural details are still preserved. Neither cornification nor pearl formation is seen. Many of the cell masses are partly or wholly calcified (see below), and the connective tissue fibers of the stroma are either incrustated with calcium or sprinkled over with granules of calcium. Cholesterin crystals are found both in the necrotic epithelial masses and especially in the degenerated stroma. The amount of fatty substance in the tumor is worthy of remark. By the scharlach R stain all the necrotic epithelial cells are, without exception, loaded with fine droplets of fat in their cytoplasm, while the entire stroma shows diffuse infiltration with large globules of fat. By differential stains (Nile blue and neutral red), the fatty acids are found to constitute the whole of the fatty material found in the epithelial masses and a large part of that found in the stroma. Neutral fat is present only in small amounts in the stroma and entirely absent in the epithelial masses. Giant cells are found in the stroma, but especially at the edges of the necrotic and calcified cell nests. Bone spicules with marrow cavities filled only with fatty tissue are found at the periphery, and sometimes entirely replacing the calcified masses. Cystic spaces are found in the tumor. The stroma at the periphery of the tumor and in the region of ossification is more cellular and vascular than elsewhere. Infiltration of lymphocytes and wandering cells is more marked at the periphery than in the central part of the growth.

CASE 5: O. P. D. No. 200170, S-19,525. C. W. K., a Chinese handicraftsman, aged 22 years, came in on June 9, 1931 with the complaint of a tumor of the right arm that had started spontaneously 2 years ago as a painless, small firm mass. It had grown in size rather quickly and was now as large as a fist. Upon examination the tumor was found to be nodular, hard, and slightly tender on pressure. Although freely movable over the muscle and bone it was adherent to the overlying skin in places. A diagnosis of fibroma was made and the tumor was excised.

Pathological Report: Specimen consists of a tumor mass having the shape and approximate size of a goose-egg, measuring 9.5 by 7.5 by 6 cm. It is heavy, stony in consistence, and sharply circumscribed, being covered on one surface by skin and on the other by subcutaneous fibrous tissue. The skin in some places can be slipped over the surface of the tumor but in other places it is adherent. Over the center of the adherent area there is in the skin a small round ulcer 3 mm. in diameter, that extends into a cavity in the substance of the tumor, from which it discharges a dirty, yellowish brown fluid. Except for the presence of this ulcer the epidermis of the skin overlying the tumor is entirely normal. The mass cuts like bone and cannot be sectioned except by sawing. The cut surface shows that the tumor, though largely in the subcutaneous tissue, also involves at certain areas the cutis — hence adhesion to the skin. The tumor tissue is grayish and yellowish white in color, sandy or chalky in consistence. The skin ulcer communicates with a cavity in the tumor 2 cm. in diameter, containing some grayish yellow, semiliquid material.

Microscopic Examination: The growth is well encapsulated by fibrous tissue. From the capsule ramifying strands of connective tissue extend into the center of the tumor forming a network, in the meshes of which lie irregular columns, masses or strands of epithelial cells. Over a few areas living epithelial cells are seen. They are small, oval, deeply staining and closely packed with vesicular nuclei, each of which contains a dense, dark blue nucleolus. These cells form almost syncytial masses with no clear cell borders. Those in the central part of the mass are more spherical and those of the periphery more cylindrical or flattened. Thus to a certain extent this tumor resembles the common basal cell epithelioma. However, only rarely can one find living cell masses without regressive changes. Almost always the central parts, and very frequently the entire masses, are necrotic, but the epithelial cells, in spite of the necrosis, retain their outline and structural details very well. Not infre-

quently one finds differentiation of the basal-like cells into pale, large, typical squamous cells, and further into cornified epithelial cells, some of which form regular pearls. The cornified pearls are practically all necrotic and are different from the epithelial pearls of the ordinary squamous cell epithelioma in that the latter consist of lamellated keratin with distinct, blue-staining nuclei, while the former have lost all nuclei and layers. Most of the necrotic masses undergo more or less complete calcification. Sometimes only one part of a necrotic cell mass is calcified and the boundary between the calcified and the uncalcified part is very sharp. Under high power distinct, fine calcium granules are seen in the cytoplasm of the necrotic cells, whose outlines are still preserved. Calcification also occurs in the stroma. The connective tissue fibers adjacent to the calcified epithelial masses become incrustated with calcium and appear as coarse, blue, wavy lines, or the hyalinized stroma between the calcified epithelial masses becomes sprinkled with deep blue, more or less uniformly-sized granules of calcium. The stroma consists of an acellular fibrillar connective tissue that is mostly hyalinized and also in places shows fairly marked edema, its fibrils being split apart by coagulated fluid that appears as fine, pink, granular material in the meshes. Cholesterin crystals are not found in the epithelial masses or in the stroma, but there is a large amount of fatty acid both in it and the degenerated epithelial cells. Neutral fat is almost entirely absent, even in the stroma, as shown by Nile blue and neutral red stains. Giant cells of foreign body type are frequent in the connective tissue, but especially numerous at the periphery of the necrotic epithelial masses, both calcified and uncalcified. Cystic spaces are present in the stroma, between it and the epithelial masses, or inside the necrotic epithelial masses, evidently as a result of necrosis and liquefaction of epithelial masses and edema of the connective tissue. The stroma of the peripheral parts of the tumor, as well as the connective tissue around it — for example in the cutis — is more cellular, more vascular, and infiltrated with more lymphocytes and wandering cells than it is in the central part of the growth, which is avascular, acellular and hyalinized. Where there is ossification the stroma has a marked cellular appearance and contains a great number of capillaries. Spicules of bone with well differentiated bone corpuscles and bone matrix are closely applied to the surface of the calcified masses. The bone forms a thin fringe

around a calcified cell mass, or replaces the outer half of the mass with a central inclusion of calcified epithelial cells whose outlines are still visible, or completely replaces the entire mass, in which case there appear also marrow cavities containing blood capillaries and loose fibrous tissue, but no myeloid or erythroblastic elements. All these various stages of ossification demonstrate clearly that bony tissue is not formed in the stroma but in the calcified epithelial masses, from outside inward. Giant cells are numerous at the edges of the calcified cell masses, but are never found around the bone.

Section of the ulcer of skin and the edge of the cavity shows just an ordinary abscess cavity lined by granulation tissue densely infiltrated with polymorphonuclear leukocytes.

CASE 6: S-20,857. Specimen received in January, 1932, from Weihwei Hospital, Honan. C. C. C., a Chinese female, aged 13 years, had had a small nodule on the forehead for 3 years, growing rapidly during the last half year. On examination a well defined, firm nodule was found partly in the skin and partly in the subcutaneous tissue. A diagnosis of sebaceous cyst was made and the tumor was removed.

Pathological Report: Specimen consists of a well encapsulated nodule, measuring 2.2 by 2.2 by 1.5 cm., situated beneath the intact skin. Consistence fairly firm. The cut surface shows whitish, chalky, very firm strands and masses separated by soft, edematous, grayish tissue.

Microscopic Examination: The growth has a well defined fibrous capsule and consists of irregular masses and strands of epithelial cells, among which no mitotic figures have been found. The epithelial cells consist in some places, of small, oval, deeply staining nuclei, each with a nucleolus. These nuclei are closely packed together so that cell borders are not seen, but in most places the nuclei are larger and the cell borders are sharply defined, in contrast with the very faintly stained or transparent cytoplasm. In some areas these latter cells are swollen with a distinctly vacuolar cytoplasm; sometimes the vacuoles even push the nucleus into an eccentric position. However, the scharlach R stain reveals that these cells contain no more fat than the unchanged small cells, although much fat in the form of big droplets is present in the stroma and in the degenerated epithelial cells. Most of the cell masses are totally necrotic, others are partially so in the center. The structural details of the necrotic cells are remarkably well preserved. Calcium is present in the ne-

crotic masses as heavy, sharply demarcated, irregular masses staining deep blue with hematoxylin, or as fine granules diffusely scattered in the cytoplasm of the individual necrotic cells, appearing as bluish dust in the low power field. The stroma consists of fibrous tissue that is more cellular in the periphery than in the center of the tumor and is in places hyalinized. Foreign body giant cells are present in the stroma, but especially along the margin of the necrotic epithelial or calcified masses. Calcification in the stroma is found either as incrustation of the fibers of the fibrillar stroma tissue, which appear as heavy, dark blue wavy lines, or as solid irregular masses or small granules deposited here and there in the stroma. Cystic spaces are found in the stroma and between the stroma and the epithelial masses. No cholesterol crystals are found in the stroma or in the degenerated epithelial masses.

CASE 7: O. P. D. No. 240400, S-21,270. W. C. H., a Chinese housewife, aged 23 years, came in on April 2, 1932, with the complaint of a slowly growing, soft mass on the left arm for about 5 months. The mass started as a small, firm, painless nodule under the skin. It ruptured spontaneously and discharged some pinkish fluid. The rupture closed up spontaneously in a week's time and the mass grew slowly but steadily, more rapidly in the last 2 months, though still painless. No similar growth elsewhere on the body. Upon examination a freely movable, globular, subcutaneous mass, soft and non-tender, 3 by 2.5 cm., was found over the anterior aspect of the left arm. The overlying skin was thin, pinkish in color and adherent, but not ulcerated. A diagnosis of parasitic cyst was made and the growth was excised.

Pathological Report: Specimen consists of a nodular mass, measuring 4.5 by 3 by 2.7 cm., covered externally by skin that is loose, wrinkled and freely movable. The inner surface of the tumor is covered by subcutaneous fat tissue. Consistence firm. On section a sharply circumscribed and encapsulated firm nodule, measuring 3 by 2 by 2 cm., is found in the subcutaneous tissue. Between it and the overlying skin there is a layer of loose fibrous tissue. The cut surface of the tumor shows whitish gray and chalky, opaque, ramifying strands and masses with some dark red streaks near the periphery.

Microscopic Examination: The tumor is completely invested by a well defined fibrous capsule and consists of irregular masses or islands of epithelial cells, between which there is a small amount of fibrous stroma moderately infiltrated with lymphocytes and in places hyalinized. The majority of the epithelial cell masses are necrotic, the cell nuclei staining pale pink like the cytoplasm. Corni-

fied pearls are frequently found in these masses. The structural outlines of the epithelial cells are well preserved in spite of necrosis. Occasionally an entire mass is seen to have undergone complete cornification, appearing as a heap of granular or structureless material poorly stained. Numerous giant cells are at the edges of the necrotic epithelial masses. Living epithelial masses are also found, especially near the capsule. They are composed of small, oval or spherical undifferentiated cells, with deeply staining nuclei closely packed without visible cell borders in the form of a syncytium. Necrosis is frequent in these masses, and usually one finds a pink necrotic central region surrounded with a blue-staining living peripheral zone. An exceptional feature of this tumor is the presence of a large number of mitotic figures in the living epithelial cells which are not found in any other tumor of the series reported. The transition from the small, dark blue, undifferentiated living cells to the pink-staining, necrotic squamous cells is either gradual or rather abrupt. Occasionally leukocytic infiltration is present in the necrotic part of the tumor. It is of interest to note that calcification of the necrotic masses occurs only to a slight extent. Most of them remain free. Calcification occurs in the form of scattered, irregular, heavy lumps in the dead epithelium, or less often as fine granulation or dusting of the cytoplasm of the epithelial cells. In the stroma calcification in the form of irregular masses or incrustation of fibrils is rare. Giant cells are present in great numbers and cholesterol crystals are not infrequently found. The Sudan III stain shows a large quantity of fat droplets in the degenerated epithelium, but not in the living cells. Large, scattered fat droplets are also seen in the stroma.

CASE 8*: S-21,311. D. T. W., a Chinese female, aged 18 years, had a stony-hard, freely movable subcutaneous tumor in the right temporal region. Four years previously she had had a boil over that region which, after a while, healed spontaneously with the formation of a very small, flat, smooth scar. In this scar, however, she later discovered a small nodule, firm and freely movable, that grew in size very slowly but continuously until at the time of excision it was 2.5 by 1.7 by 0.8 cm.

Microscopic Examination: The tumor mass consists of irregular masses of epithelial cells embedded in a fibrous stroma that is in a few places cellular but in most places dense, collagenous or hyalin-

* Only a paraffin section slide of the tumor growth was received on April 7, 1932, from Dr. H. C. Pai of the Mukden Medical College, with the above brief clinical note.

ized. The block represents only a small portion of the tumor and only at one side is the tumor tissue surrounded by a thin layer of fibrous capsule and some fibro-adipose tissue. Beneath this ill defined capsule there is dense lymphocytic infiltration of the tumor stroma. The epithelial masses are all totally necrotic with pink-staining nuclei, and all show calcification of varying degrees, except one or two. No living epithelial cells are present. In the necrotic epithelial cell masses cornified pearls are frequently found, thus indicating their squamous nature. The outlines of the cells are still recognizable, although they are necrotic. Calcification occurs from mere dusting or fine granulation of the cells to a massive, deep blue deposit obscuring entirely the cellular structure of the masses. Giant cells of foreign body type are seen at the periphery of the calcified masses, many of which are completely or partly surrounded by a thin fringe or a thick layer of typical bone. Sometimes the calcified mass is almost completely replaced by bone. The structure of the epithelial cells of the calcified masses is well preserved, even when their outer zone has been changed into well formed bone. Calcification of the stroma occurs chiefly in the form of incrustation of the hyalinized fibrils. Giant cells are occasionally found in the stroma.

CASE 9: S-21,946. W. M. Y., a Chinese female, aged 16 years. A firm, freely movable, subcutaneous mass, 0.5 cm. in diameter, over posterior lateral aspect of left arm, somewhat adherent to the overlying skin, of more than 3 months duration. Tumor excised.

Pathological Report: Specimen consists of 3 broken pieces of tumor tissue measuring 0.5 cm. each in length. Two of them externally are partly covered by epithelium and partly by fibrous tissue. The third piece is covered by adipose and fibrous tissue. Consistence firm. Cut surfaces show yellowish, opaque chalky strands embedded in a whitish tissue mottled with a few small dark red areas and encapsulated on the free edge of epithelium or fibro-adipose tissue.

Microscopic Examination: The tumor tissue consists of irregular strands or masses of totally necrotic, pink-staining squamous epithelium with areas of cornification and pearl formation. The structures of the squamous cells are preserved in spite of necrosis. Small, living epithelial cell masses, few in number, are found here and there, consisting of dark blue-staining, small, oval nuclei, each with a single nucleolus, closely packed in purplish-staining cytoplasm in the form of a syncytium without clear cell borders. The central

parts of these masses practically always show necrosis. Oftentimes necrotic masses have only a fringe of living cells at the periphery or to one side. The transition between the living and necrotic cells is gradual, much more gradual than in the previous cases. The dark, small, closely packed nuclei differentiate into larger, paler and less closely grouped oval or rounded nuclei, each with one and sometimes two nucleoli, and as they differentiate the cytoplasm increases in amount and the cell borders become definite. Masses of these relatively well differentiated, living squamous cells are present between the small typical living cells and the necrotic squamous epithelium, and constitute a rather special feature of this tumor because, in the previous cases, these cells were very few or not seen. Toward the necrotic part of the tumor the nuclei of the cells become pyknotic until in the necrotic area they stain a pale pinkish color of the same degree as the cytoplasm. The fibrous stroma is rather cellular and contains foreign body giant cells. Numerous areas of calcification are seen in the necrotic cell masses. The calcium is seen under higher magnification as irregular granules or fine dusting in the cytoplasm of the cells. There is no bone formation.

CASE 10: O.P.D. No. 246613, S-22,034. Y. S. F., Chinese female, a student, aged 20 years, came in on July 1, 1932, with the complaint of a tumor in the left side of the neck of 8 to 9 years duration, and of slow growth. Examination showed a freely movable, subcutaneous, firm, slightly tender tumor nodule the size of a pea, situated behind the left ear lobule in the mastoid region. A diagnosis of calcified sebaceous cyst was made and the growth excised.

Pathological Report: Specimen consists of an irregular nodule of bony-hard consistence, measuring 1.2 by 0.8 by 0.4 cm. The external surface is smooth and is covered by congested, purplish, fibrous tissue. The mass could not be sectioned before decalcification. After that the cut surface revealed interlacing or anastomosing strands of yellowish brown and grayish, opaque chalky tissue.

Microscopic Examination: The tumor is enveloped by a well defined, thin, fibrous capsule, and consists of irregular anastomosing strands and masses of necrotic and mostly calcified squamous epithelial cells, the structural details of which are still fully recognizable in spite of necrosis and calcification. No epithelial pearls are found and no living epithelial cell masses are present. Calcification of the necrotic masses is denser and heavier in the periphery of these masses where coarse, dark blue granules of calcium are visible

in low power, and much lighter and finer granules are seen in the central parts of the masses, where fine dusting of the cytoplasm of the individual cells is visible under high power. The stroma consists of cellular fibrous tissue that contains a large number of blood capillaries and is in places infiltrated by small lymphocytes. Calcification of the stroma in the form of incrustation of its fibrous fibrils (which appear as dark blue wavy lines) is also present. Foreign body giant cells are found at the edges of the calcified masses and also free in the stroma. Characteristically shaped cholesterol crystals are found deposited both in the calcified epithelial masses and in the stroma. Typical bone tissue is found replacing the periphery of the calcified masses, varying from a very thin fringe to a thick deposit. Frequently such bone tissue includes calcified epithelial cells or contains marrow cavities filled with vascular fat tissue but without hematopoietic elements.

DISCUSSION

From the description of our cases and of those reported in the literature it is clear that the calcified epitheliomas of the skin form a group of tumors that are clinically and pathologically characteristic. The benign behavior of the majority of them and their invariable tendency to undergo necrosis and calcification are the distinguishing features. They resemble the basal cell epitheliomas in many respects. Both consist of small, oval, deeply staining and closely packed cells, and both have the same type of distribution over the body, *i.e.* they are most frequently found over the head and neck. On the other hand, they are different in many important respects. The calcified epithelioma has a complete and well defined capsule, is freely movable and does not ulcerate or invade the overlying skin. The basal cell epithelioma has no capsule, is not freely movable but is locally invasive and produces a characteristic ragged ulcer (rodent ulcer). The cells of the calcified epithelioma rarely show mitosis and are grouped into round or elongated masses that are sharply demarcated from the stroma by a distinct basement membrane; the cells of the basal cell epithelioma, on the other hand, are apt to show frequent mitosis and grow in infiltrative, net-like (reticulated) strands. The cells of basal cell epithelioma only occasionally differentiate into squamous epithelium with cornification or pearl formation, whereas those of the calcified epithelioma almost

invariably differentiate in this manner. The calcified epithelioma always contains areas of necrosis and calcification, the basal cell epithelioma only rarely. The age incidence is different; the calcified epithelioma occurs in youth, the basal cell epithelioma occurs characteristically after 40. With basal cell epithelioma recurrence after extirpation is not uncommon; with calcified epithelioma it is extremely rare.

The rounded or ball-like contour and the subcutaneous position of the tumor with occasional traumatic ulceration of the overlying skin (following stretching by the expansile growth of the tumor) and discharge of necrotic epithelium have led to the clinical diagnosis of sebaceous (epidermoid) cyst, and the presence of bone tissue may cause the false impression of its being a dermoid or mixed tumor, but the anatomical structure is so different that it is superfluous to enter into a differential diagnosis here. The finding of typical squamous cells besides the small dark cells, and of the cornified pearls, should at once reveal to the observer the true nature of an epithelioma, although, when these characteristics are not evident, as in the totally necrotic or heavily calcified growths, confusion with other tumors, *e.g.*, endothelioma, sebaceous adenoma, and so on, may arise. The presence of fat in the epithelial cells and the deposit of cholesterin crystals in the necrotic epithelium have been taken, for instance by Eve, as an indication of the sebaceous origin of these tumors, but there is no ground for such an opinion since in any degenerating tissue the occurrence of fat and cholesterin is very common.

The different steps in the laying down of bone can be readily followed in our tumors. First the cell mass becomes necrotic, then calcified. The calcium is later replaced by new bone from the periphery inward. That the bone is formed largely as a result of ossification of the calcified masses is shown by the fact that the bony tissue has the same general shape or contour as the calcified masses, and that in the irregular beams, strands or trabeculae of bone tissue seen in the sections central inclusion of calcified epithelial cells is usually found, and also that all stages of transition from a calcified cell mass with just a narrow fringe of bone to one completely replaced by bone can be followed readily in the sections.

It is a very significant fact that 7 of our 9 cases (1 case with incomplete history not counted) are tumors which, although freely mov-

able over the deeper tissues, are adherent to the skin. Since these tumors are sharply encapsulated and do not invade the overlying skin, their adhesion to the latter can be explained only by the assumption that they are originally situated in the skin, or rather they are derived from the skin or its appendages.

There are certain features described in the literature that are not found in any of our cases and these are granulation tissue and myeloid tissue, obliterative changes in the arteries, which are looked upon as the cause of the necrosis, and myxomatous or sarcomatous degeneration of the stroma.

In 4 of our cases the peripheral portion of the tumor is definitely much more cellular and vascular than the central portion, and in 2 of these and 2 more from the rest of our cases the peripheral or subcapsular zone is moderately or densely infiltrated with lymphocytes and wandering cells. Similar findings have been made by Henzi.

Cystic spaces are found in the majority of our cases, and sometimes they are filled with fluid. These arise probably in the manner already mentioned above. Many of the spaces are irregular in shape and distribution and are empty and without fluid contents. Apparently they are artefacts.

As to whether trauma is the exciting cause of the tumor growth or not, the clinical histories of most of our cases do not give us satisfactory information. Only one case (Case 8) gives the previous history of a furuncle. It is possible in this case that during the healing of a furuncle the regenerating epithelium was displaced and later gave rise to a tumor. The common explanation of the traumatic origin of an epidermoid (sebaceous) cyst or calcified epithelioma, however, is that the mechanical injury dislocates or carries into the subepithelial tissue a bit of the epithelium that later grows into a tumor. It is hardly conceivable that trauma of this kind can be the chief cause, since these tumors are found not on the parts of the body that are more exposed to injury, but on the head and neck where trauma is relatively less frequent.

Although no good follow-up histories are available in our old operated cases, lack of recurrence after complete removal has been recognized in the literature as a feature characteristic of most of these tumors. Anatomically their non-malignant nature is evidenced by the constant possession of a well formed capsule, the

sharp demarcation of the epithelial masses and the scarcity of mitotic figures, together with the free-movability, the slow and expansile growth and the lack of ulceration. In view of the presence of cases of recurrence in the literature, however, the prognosis of these tumors should be guarded, even though the benign nature is apparent in most of them.

SUMMARY

Ten cases of calcified epithelioma of the skin examined in the pathological laboratory of the Peiping Union Medical College are reported. These tumors form a distinct group of neoplasms that are anatomically and clinically well defined. They are circumscribed, well encapsulated growths beneath the skin, consisting of lobulated epithelial masses with a network of usually hyalinized fibrous stroma. The epithelial cells are small, oval, deeply staining and closely packed, and have a marked tendency to undergo necrosis, calcification and ossification. A study of our 10 cases and the 116 cases collected from the literature indicates that they are distributed most frequently on the head and neck and occur usually among the younger individuals. The large majority of these tumors are benign, but a few cases of recurrence following removal have been recorded.

The author wishes to express his appreciation to Dr. C. H. Hu for many valuable suggestions and aid in the preparation of the above article.

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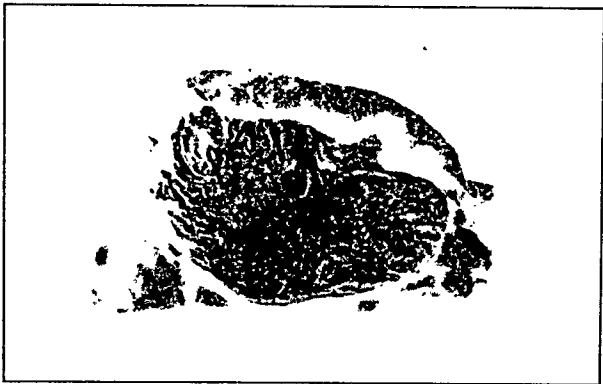
DESCRIPTION OF PLATES

PLATE 72

- FIG. 1. Cut surface of the tumor in Case 5. Natural size. Note its sandy or gritty appearance and the fact that the growth is enveloped by intact skin on one side and by a thin fibrous capsule on the other.
- FIG. 2. Cut surface of the tumor in Case 7. Natural size. The tumor is subcutaneous, covered by skin on one side and by a thin fibrous tissue capsule on the other. Note the characteristic appearance of the cut surface.
- FIG. 3. Tumor in Case 6. Natural size. Note relation of overlying skin to tumor.
- FIG. 4. Tumor in Case 4. Natural size. Note encapsulation.
- FIG. 5. Representative field of the tumor in Case 5, showing the general character of the growth. Note the alveolar arrangement of the epithelial masses. The living cell masses are stained deep blue by hematoxylin, appearing black in the picture. Practically all of them show either beginning or already extensive necrosis at the center. The necrotic epithelial masses are stained lightly with eosin and appear gray in the picture. $\times 13$.
- FIG. 6. A living epithelial cell mass from the tumor in Case 5. Note the small, oval, closely packed, deeply staining basal-like cells and their transition into squamous cells. $\times 155$.



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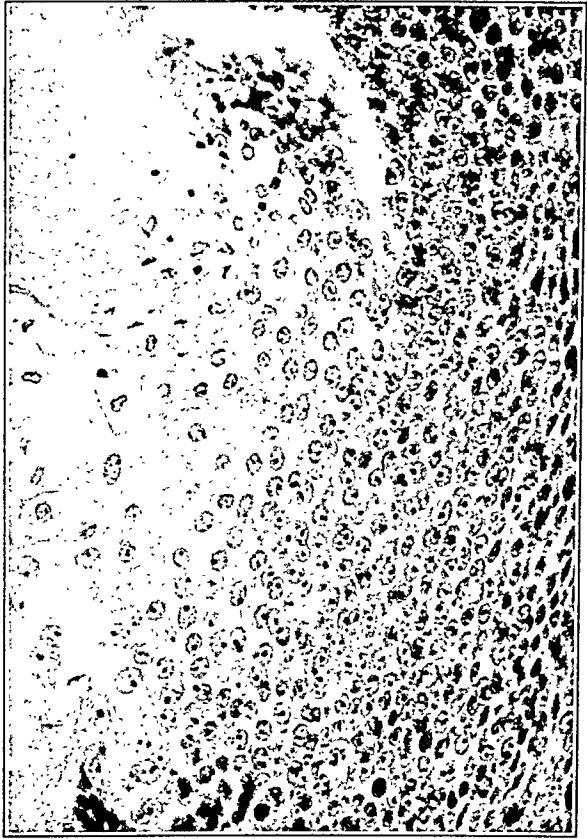


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Ch'in

Calcified Epithelioma of Skin

PLATE 73

- FIG. 7. Epithelial pearl surrounded by a thick mantle of squamous cells. Basal-like cells at the periphery. $\times 155$.
- FIG. 8. Tumor in Case 5 showing giant cells at the periphery of a mass of degenerated squamous cells whose nuclei are no longer stained with hematoxylin but stained pink with eosin, yet whose structural details are well preserved. The stroma is slightly infiltrated with lymphocytes. $\times 155$.
- FIG. 9. Tumor in Case 5 showing the deeply staining areas of calcification in the degenerated epithelial masses and calcium incrustation of the fibers of the hyalinized stroma. $\times 70$.
- FIG. 10. Tumor in Case 2 showing spaces previously occupied by cholesterol crystals in the degenerated epithelial cell mass with large numbers of fine or coarse granules of calcium deposit giving a punctate appearance to the cells. $\times 155$.
- FIG. 11. Tumor in Case 4 showing calcium deposit in the totally degenerated epithelial masses and bone trabeculae, many of which include degenerated and calcified epithelium in their centers. No living epithelial masses are present in this tumor. Note the sharp demarcation of the tumor and its possession of a fibrous capsule at the right hand field. $\times 13$.
- FIG. 12. Tumor in Case 4, showing the ossification of a calcified epithelial mass from periphery inward. $\times 70$.



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12

Calcified Epithelioma of Skin

SARCOMATOID METASTASES IN THE LYMPH NODES DRAINING A PRIMARY CARCINOMA WITH A SARCOMATOID STROMA *

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INTRODUCTION

The case I wish to report may be allotted to the category of the carcinosarcomas. I hesitate to do so for reasons that are made clearly evident in the text to follow. The remarkable point of interest in this case is, primarily, the interpretation of the sarcomatoid metastases in the lymph nodes draining a primary carcinoma with a sarcomatoid stroma.

The literature abounds with cases rightly and wrongly described as mixed tumors of the breast, thyroid and uterus. Occasionally, though rarely, two tumors are really present together, *i. e.* carcinoma and sarcoma. A true double tumor of such nature may be met with occasionally in the human subject. Great care must be exercised in interpreting appearances, as occasionally cancer cells may be diffusely arranged and even spindle-shaped. Cancer cells readily and frequently resemble mesoblastic elements, whether from pressure or from lack of differentiation, so that it is usually possible to raise a doubt as to whether the supposed sarcomatous elements are really such or merely deceptively altered epithelial cells.

I shall attempt to establish, therefore, that the sarcomatoid stroma in this case is not modified epithelium and that the spindle cells are of true connective tissue origin. This conclusion has been facilitated by the selective trichrome blue stain of Masson, and rechecked by Mallory's phosphotungstic acid hematoxylin stain in the histological study of the case.

Before describing this case I shall briefly review some of the controversies held on the subject of mixed carcinosarcomas. The state of utter confusion and the various opinions existing permit this further study.

* Received for publication March 4, 1933.

We are not concerned in this discussion with the primary mixed tumors. That certain tumors originate through neoplastic growth of both stroma and epithelial cells in a tissue complex is conceded. It is difficult to determine which of the carcinosarcomas originate in this manner.

We are concerned here with secondary mixed tumors, that is, sarcoma arising from the stroma of carcinoma. This, of course, is not universally tenable. Several theories have been advanced, but the main schools of thought are:

- (a) Sarcomatous transformation of the connective tissue stroma of carcinoma (Haaland,¹ Ehrlich²).
- (b) Spindle cell metaplasia of the epithelial cells. The altered epithelium may simulate a sarcoma in appearance (Ewing³).
- (c) These sarcomas are nothing but an exuberant proliferative growth of ordinary connective granulation tissue (Orth⁴).

It was a most surprisingly new fact, discovered by Ehrlich and Apolant,⁵ that the stroma in certain transplantable mouse carcinomas gave rise to a new and distinct kind of tumor tissue, *i. e.* a transplantable sarcoma. In succeeding generations the sarcoma ousts the carcinomatous elements and entirely supplants them. Ultimately a pure sarcoma is obtained. This phenomenon has been observed by many others.

Haaland agrees with the opinions of Ehrlich and Apolant that the development of sarcoma is explicable as a new or altered form of specific reaction to influences proceeding from the carcinoma cells. This enhanced reaction on the part of the connective tissue, progressing to sarcoma, depends not only on the stimulating influence proceeding from the carcinoma but also on the individual response of the host.

The question arises whether these sarcoma-like tumors are true malignant newgrowths and not mere infectious granulomas or granulation tissue around the carcinoma alveoli. Orth, commenting on Lewin's case,⁶ questioned the existence of sarcoma. He thought sarcoma was not really present but rather a growth of connective granulation tissue. The presence here and there of spindle cells is also a feature of ordinary proliferations of connective tissue.

Ehrlich and Apolant rejected the possibility of the new tumors being granulomas since metastases in the pulmonary artery by

emboli of tumor cells continued growing. The presence of collagen fibrils between the cells in intravascular secondary nodules proved the connective tissue origin of the cells.

Murray ⁷ has strongly substantiated the belief that sarcoma arises from the stroma and not from altered cancer cells. Haaland, under high magnification, observed in the cytoplasm of these rapidly growing, spindle-shaped cells characteristic fine fibrils (Mallory's fibroglia fibrils) like those found in young connective tissue (Maximow⁸). Other fibrils were found outside the cells, giving all the reactions of collagen. The production of collagenous fibrils and the presence of fibroglia fibrils in the cells proved their origin from fibroblastic elements of the connective tissue. In my opinion this justifies calling these tumors true sarcomas. Murray dismisses the idea of the possibility of the sarcomatous cells being derived from carcinoma parenchyma since these cells present all the characteristics of true connective tissue cells as described above. There exists no analogy for a process of metaplasia that can transform typical epithelial cells into equally typical connective tissue cells. It is, however, generally admitted that the epithelial cells of the neuroglia have the faculty of producing a network of fibrils.

Dorsch ⁹ in 1896 reported a case of carcinosarcoma. He concluded that sarcoma arose through stimulation of connective tissue by the epithelial cells. This curious transformation of the stroma in cancers has been observed by many others. Oertel ¹⁰ classifies carcinosarcomas into several categories, but says that the "genuine carcinosarcoma is the sarcoma that develops out of the stroma of an already existing cancer." The stroma acquires new characteristics and begins to proliferate. Occasionally it assumes neoplastic characteristics. In this manner carcinosarcoma results (Masson¹¹). Mallory ¹² favors the view that in epithelial tumors (especially skin and mammary gland) the cells can chemically or otherwise directly influence and determine the amount of the stroma. The growth is at times so active that it is not unusual to find fibroblasts in mitosis proliferating actively and producing many collagen fibers.

On the other hand Ewing (in answer to Ehrlich and Apolant, Haaland, and others) finds it difficult to conceive how an original carcinoma can be made to yield all its growth energy to normal stroma cells and completely retire from the field, yet this anomalous result has been reached in the pure sarcoma developing from mouse

carcinoma. In lower animals the ready response of fibroblasts to various stimuli renders more acceptable the belief that transplanted stroma occasionally assumes neoplastic properties during a series of transfers, but does not prove the existence of such a process in the course of tumor growth in man. Ewing believes that the influence of transplantation renders these observations inapplicable to human tumors and that several uncertainties still surround the interpretation of this remarkable change in structure.

Ewing feels the correct interpretation of the carcinosarcoma structures in man and lower animals is a matter of difficulty. The chief source of these structures is the transformation of epithelial cells into spindle cells. This change is of widespread occurrence in epithelial tumors, and is facilitated by rapid growth, inflammatory exudate and relief from pressure. He has seen pure spindle cell metastases in epithelioma of the lip, adamantinoma, and so on. Krompecher¹³ believes that the carcinosarcoma is merely a carcinoma; its spindle-shaped cells being epithelial in spite of its morphological resemblance to fibroblasts. His studies on the metaplasia of squamous epithelium have an important bearing on this subject.

On examining several of Woglom's cases purporting to show the sarcomatous transformation of stroma cells Ewing concluded that the spindle cells were derivatives of epithelium. This view has been enthusiastically accepted by many. Gessner¹⁴ refers to all so-called carcinosarcomas of the uterus as epithelial in origin. Meyer¹⁵ believes that sarcomatous transformation of carcinomas has not been demonstrated.

Helwig¹⁶ reviews twenty-four cases of reported mixed tumors of the breast and concludes that it is impossible to draw any definite conclusions as to whether the tumors are true carcinosarcomas, or if metaplasia has occurred with a gradual transition from a carcinoma into a sarcoma-like structure. In his own case, which simulated histologically a picture of a carcinoma and giant cell sarcoma, he is convinced that it is a carcinoma with a sarcomatous metaplasia of epithelial cells.

Out of a considerable number of so-called mixed tumors of the uterus Herxheimer¹⁷ found only five that could withstand critical analysis. Schmorl's case of thyroid adenoma, recurring as carcinoma with sarcomatous stroma and with sarcomatous metastases,¹⁸ is

doubted by Ewing. In thyroid tumors the form of epithelial cells is subject to wide variations, a fact that renders Schmorl's interpretation of his carcinosarcoma very questionable. Definite association of sarcoma with carcinoma of the breast has been reported by a few observers, but in several such cases Ewing has not been satisfied that the spindle cell areas were not modified epithelium. Nevertheless, Mason and Wells,¹⁹ in reporting their case of carcinosarcoma, assert that it is not a carcinoma with altered epithelial cells since the carcinoma portion metastasized to the cervical lymph nodes and the sarcoma portion to the kidney.

Indeed, many observers agree that in rare cases we do not know whether a given cell group originates in epithelium or not, since the facts do not justify such definite distinctions between epithelial and certain other tumors. Cohn,²⁰ in reviewing this subject, maintains that our available means for differentiating epithelial and sarcomatous elements are still so defective that a positive proof cannot usually be offered to support a diagnosis of mixed sarcoma and carcinoma, especially in cases of advanced spontaneous tumors.

Finally, it is to be remembered that in some forms of basal cell carcinoma the epithelial cells may have an indifferent spindle-shaped contour, often elongated, even fibrillar (Kaufmann²¹). Cancers arising from hair matrices often show large masses of spindle cells running in bundles, frequently produce numerous long and coarse fibrils, and may suggest spindle cell sarcoma (Mallory¹²). Kaufmann,²¹ in quoting Krompecher, emphasizes the possible variability of ectodermal cell proliferation, especially the transition of ectodermal into apparently mesenchymal structures. Masson²² in his Atlas of Cancer shows beautifully colored illustrations of mixed tumors of the parotid in which the ectodermal tumor cells demonstrate their capacity to assume the appearance of mesenchymal tissue.

In view of the interest in these tumors the following case is reported.

REPORT OF CASE

Clinical History: Mrs. A. L., aged 74 years, was admitted to the Notre Dame Hospital Dec. 15, 1931 with a superficial, ulcerated tumor 3 cm. by 2.5 cm., situated in the skin of the left mammary fold, medial to the nipple line and definitely independent of the mammary gland itself. It was quite hard and had

an inflammatory zone about it. The tumor was removed and sections made for histological study. A diagnosis of carcinosarcoma was made.

At that time lymph nodes in the axilla were not palpable. Radical excision of the breast was advised but the patient refused. The biopsy incision healed satisfactorily and the patient left the hospital in good condition. Physical examination revealed nothing of importance.

Two months later the patient noticed a swelling in the left axilla. This gradually enlarged and she finally returned to the hospital Sept. 9, 1932 (almost eight months later). At operation one lymph node 2.5 by 1 cm. in size and another 1.5 by 1.5 cm. were removed and sections made for histological study. The lymph nodes were diagnosed as fibrosarcoma.

The patient has been under observation and has been well and free from further recurrences.

MICROSCOPIC DESCRIPTION OF THE PRIMARY TUMOR

The tumor is spherical in form and for descriptive purposes it may be divided into three zones of a circle.

Outer Zone: Epithelioid processes growing centrifugally from the center of the tumor invade the epidermis and the underlying dermis. The epidermis is locally ulcerated. The cancer growth is of the higher type, preserving some of the attributes of its epithelial origin. A few prickle cells can be seen in some of the strands invading the stroma. Here and there an abortive attempt at cornification by individual cells is observed. The cancer cells are polygonal in shape, well formed, evenly stained, showing relatively few mitoses. Most of the lymphatics are plugged with large epithelioid processes growing transversely, although some of the lymphatics are empty and free. The connective tissue stroma varies considerably in different areas. For the greater part the stroma is quite delicate and not very cellular. Toward the periphery at each end the connective tissue is quite mature, displaying very few cells and a well stained collagenous material. At other points the stroma forms a loose connective tissue of large cells regularly ensheathing the epithelial strands and encasing empty lymphatics. There is a marked inflammatory reaction, particularly near the ulcerated zone, in the form of many plasma cells, some lymphocytes and a few eosinophils. The blood vessels are free from invasion, although a few show endothelial proliferation within the lumen.

Mid Zone: The epithelial strands are not as numerous as in the outer zone, and show some parenchymal degeneration. Accompanying the degeneration within these strands is a markedly abundant

stroma that has become frankly sarcomatoid in character. It now shows increased cellularity and numerous cell divisions. The cells are larger and usually elongated, though varying in shape. This rapidly proliferating connective tissue forms broad bands of spindle-shaped cells surrounding the cancer alveoli (almost in halo fashion) and separates them from one another. In other areas of this zone a further and greater activity of the stroma is observed. Here, the cellularity and proliferating quality of the stroma is sarcomatous in nature. The cells are large, rapidly dividing and show abundant fibroglia fibrils and collagenous fibrils between the cells.

The fibroblastic proliferation is here characterized by an immature manner of growth, proliferating without restraint, showing a number of monstrous cells with bizarre mitotic and multipolar figures and nuclei of extraordinary size. Hyperchromatic and hypochromatic nuclei are frequent. The amount of cytoplasm varies greatly and a marked polymorphism prevails. The cells are much larger than usually seen in granulation tissue. Here the stroma reaction is so intense that it overrides and eats into the cords of cancer cells so that within this stroma only scattered epithelial cells are observed. These epithelial cells are unlike the neighboring stroma cells which are pale, irregular in shape and contour, and with definite fibroglia fibrils (as seen by the trichrome stain).

Central Zone: The epithelial strands are very much necrosed. The collagen fibrils laid down by the fibroblasts are thick, well stained and sclerotic. Atrophic and necrotic changes are observed here in the epithelial alveoli surrounded by this sclerotic tissue. These changes proceed from the center of each alveolus toward its periphery, often leaving only a single layer of cancer cells close to the stroma tissue, while the rest of the alveolus is necrotic. In the center the epithelial strands are almost wholly sclerosed with calcareous deposits in evidence. The stroma has become hyalinized and acellular.

MICROSCOPIC DESCRIPTION OF THE LYMPH NODES

In the larger lymph node at one end there still remains the normal structure of lymphoid tissue. Everywhere else a fibrosarcomatous tissue replaces the lymph node tissue and its capsule, and also invades the pericapsular fat. The hilum seems to have been first at-

tacked. The areas neighboring the hilum are sclerotic. The direction of the growth of the tumor is from within outward, following the sinuses. There seems to be a transformation of endothelial cells into fibroblasts *in situ*. Epithelial strands, or cells resembling those of the primary tumor, cannot be found but mixed among this sarcomatoid structure are many polymorphous cells. The origin of these large polymorphous cells is probably from the mesenchymal tissue of the lymph node.

At one point in the periphery of the lymph node, an afferent lymphatic vessel is seen, within which free epithelial cells are floating in the lymph. This is the only definite trace of epithelial cells, but this is evidently a later stage of the picture. The cells lie free and unattached, unable to enter the lymph node, the lymphatics of which have been blocked by the fibrosarcomatous replacement of the tissue. Most of the other lymphatics are empty, some are filled with lymph, while others show a marked endothelial proliferation. The blood vessels do not show any involvement other than endothelial proliferation. For the greater part this fibrosarcomatous tumor consists of interlacing bundles of spindle cells. Between the individual cells fine collagen fibrils are to be seen. With higher magnification fine fibrils are found in the cytoplasm of the cells (fibroglia fibrils, Mallory).

The smaller nodule, round in shape, shows traces of its lymphoid structure. The lymph node is practically completely replaced by the fibrosarcomatous tissue. The capsule is entirely destroyed and the surrounding fat is invaded to a greater degree than in the one previously described.

DISCUSSION

1. These spindle-shaped cells are not the result of epithelial metaplasia. Transition figures between these cells and cancerous epithelial cells are not to be found. Frequently many degenerating cancer cells isolated in the sarcomatoid stroma are seen. Sarcomatous transformation of the stroma cells results (*a*) when in contact with epithelial cells which, moreover, are degenerating or dead, and (*b*) when surrounding lymphatics, whether they contain epithelial cells or not.

It appears that the transformation and the multiplication of the stroma cells are determined by some substance contained by the

cancer cells. When their degeneration occurs this substance is liberated and acts directly on the fibroblasts whether it be *in situ* or after being absorbed by the lymphatics. As we shall see later, this is the only explanation that allows us to understand the sarcomatoid metastases in the lymph nodes, their structure and manner of growth. If these spindle-shaped cells are but altered epithelial cells their appearance in the lymph nodes is not altogether surprising. It would be difficult to explain the fact that the lymphatics in the primary tumor were plugged with well stained, polygonal-shaped epithelial cells, and that nowhere could a lymphatic vessel be found containing spindle-shaped cells.

Histologically these spindle-shaped cells behave like fibroblasts and possess the same selective staining properties. Even if the general appearance and behavior of the rapidly growing spindle-shaped cells would not allow any conclusions as to their origin, other points might permit a decision as to which elements they have arisen from. Using Masson's trichrome blue technique, fibroglia fibrils stain red and collagen fibers stain blue. Under high magnification characteristic, fine, red fibrils are seen in the cytoplasm, like those described by Mallory and similar to the fibroglia found in young connective tissue (rechecked by Mallory's phosphotungstic acid hematoxylin). These red fibrils are found intracellularly following the prolongations of the cell; other such fibrils are found extracellularly. Between the cells collagen fibrils staining blue are numerous. The cells behave like fibroblastic tissue, forming a scaffold of interlacing bundles of spindle cells. As the collagenous material laid down by these cells becomes excessive the cells themselves become scarce.

The presence of fibroglia fibrils in the cells, the production of collagenous fibrils outside the cells and their behavior, aside from their general appearance, prove their origin from fibroblastic elements of the connective tissue. It is, therefore, justifiable to dismiss the possibility of epithelial metaplasia and to suggest an explanation as to the histogenesis of the spindle cells.

2. It is necessary, in dealing with mixed tumors, to establish whether they are primarily or secondarily mixed, as previously pointed out. Here the primary skin tumor is not primarily a mixed tumor. The cancer and sarcomatous stroma are not proliferating together. The cancer only invades and provokes the production of a stroma at the expense of the local connective tissue. The tumor is

secondarily mixed. It is only at some distance from the zone of invasion that the stroma assumes sarcomatous characteristics.

3. How can the appearance of fibrosarcomatous tissue in the lymph nodes be accounted for in this case? Generally, sarcomas do not metastasize along lymphatics. Lymphosarcomas are the only sarcomas that frequently travel by the lymphatics and the origin of these tumors within the lymph nodes fully accounts for this tendency. Melanosarcomas are epithelial in origin (Masson), which accounts for their specificity in affecting lymph nodes. Most statistical reports of lymph node invasion in sarcoma are of doubtful value owing to the uncertainty connected with the diagnosis of sarcoma. Most of the tumors reported as sarcomas of the testis and thyroid are probably epithelial tumors and commonly invade the lymph nodes (Ewing).

On the other hand it has been observed that sarcomas, although having metastasized by the blood stream, nevertheless set up irritative endothelial proliferation in the neighboring lymph nodes. Usually the soluble products absorbed by the lymphatics from neoplastic nests (cancerous or sarcomatous) initiate only an endothelial hyperplasia, followed by fibroblastic transformation and sclerosis. In this case the stroma cells of the lymph node bathed in the lymph that had absorbed the products liberated by the epithelial cells in the primary tumor. A sarcomatoid transformation of these stroma cells resulted (just as there was a sarcomatoid transformation of the stroma around the lymphatics at the edge of the primary tumor).

4. And lastly it is necessary to prove that in the lymph nodes small remains of carcinoma still were not present, inciting the connective tissue to an actively proliferative, granulomatous growth, thereby explaining the appearance of a presumably sarcomatoid tissue in the lymph node.

Metastases of cancer growth can lead to an abundant stroma corresponding more or less closely in amount to the original tumor, even in a tissue containing as little connective tissue as the bone marrow. The behavior of the stroma may become so active that it is often difficult to distinguish sharply between those tumors whose stroma is clearly not neoplastic and those in which it constitutes a part of the tumor and which must be regarded as mixed tumors. In this case there may have been an epithelial metastasis in the lymph node with a resulting sarcomatoid transformation of the stroma. De-

generation of the cancer cells followed, allowing the sarcomatoid tissue alone to persist and to proliferate actively. This is possible, but not probable. That the products liberated by the degenerated epithelial cells in the primary tumor were absorbed by the lymphatics and directly influenced the stroma reaction on reaching the nodes is the preferable view-point. This point is important. In the irritative endothelial hyperplasia of lymph nodes the toxic products entering the afferent lymph vessels of the nodule drain along the sinuses and reach the hilum. These products first initiate this endothelial hyperplasia at the hilum; this reaction then radiates outwardly toward the cortex, if the irritative stimulus is great or prolonged. In this case a similar process has occurred (the hilum having been first attacked, as previously pointed out in the microscopic description of the lymph nodes) with not only a fibroblastic transformation of the endothelial cells lining the sinuses, but also a sarcomatoid transformation. This is what has occurred here, rather than a neoplastic invasion, for if this had been so, evidence would exist in the cortex of the lymph node near the entrance of the afferent lymph vessels. The changes in the lymph node in this case are comparable to the usual irritative endothelial hyperplasia. This permits the belief that neither an epithelial nor a sarcomatous metastasis has occurred, but rather a sarcomatoid transformation *in situ* similar to that occurring around the cancer alveoli of the primary tumor.

It will be interesting to know if this sarcomatoid tissue of the primary tumor and the lymph gland is truly sarcomatous, that is to say, now possesses the autonomy of newgrowth with its characteristic independent proliferative qualities, or if it is only sarcomatous in appearance, due to especially irritative products originating from the cancer cells. In this regard the further course of the disease alone can inform us. If the tumor is truly sarcomatous, then metastasizing by way of the blood stream and not lymphatics is probable (*cf.* case of Vadon *). If the tumor is pseudosarcomatous, the appearance of new fibroblastic tumors will be dependent on carcinomatous metastases or a local recurrence.

* Professor Masson relates the following incident concerning Vadon, an interne in one of the hospitals of Paris. While aspirating the fluid from a cystic mass of the breast he accidentally stuck his hand with the aspirating needle. The mass proved to be carcinoma at later examination. In the meantime a growth in his hand developed at the point of injury. This growth was purely fibrosarcomatous. This generalized by way of the blood stream and he died. The metastases proved to be pure sarcoma.

SUMMARY AND CONCLUSIONS

1. A case of sarcomatoid metastases in lymph nodes draining a primary carcinoma with a sarcomatoid stroma is reported.
2. The usefulness of Masson's trichrome blue stain, particularly in the histological study of the fibroglia of fibroblasts, is here emphasized.
3. The possibility of epithelial metaplasia is discussed.
4. An analogy between this case and the experimental tumors of Ehrlich, Haaland, and others is assumed.
5. The dividing line between mixed tumors and tumors whose stroma is not clearly neoplastic is difficult to determine. In this case further metastases by way of the blood stream alone will decide.
6. The occurrence of sarcoma in the lymph nodes in this case may be explained on the basis of absorption of products from degenerated epithelium by the lymphatics. This initiates a stroma reaction in the lymph nodes which may become neoplastic in character, depending on the tissue response of the host and the irritative quality of the absorbed products liberated by the degenerating cancer cells.

I am indebted to Professor Masson for his kindly interest, guidance and encouragement in this work, and I am also indebted to Dr. L. C. Simard for the preparation of the photomicrographs.

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DESCRIPTION OF PLATE

PLATE 74

FIG. 1. Primary tumor. Carcinomatous plug of epithelioid cells. The surrounding connective tissue stroma is quite normal. Note the marked inflammatory stroma reaction. $\times 600$.

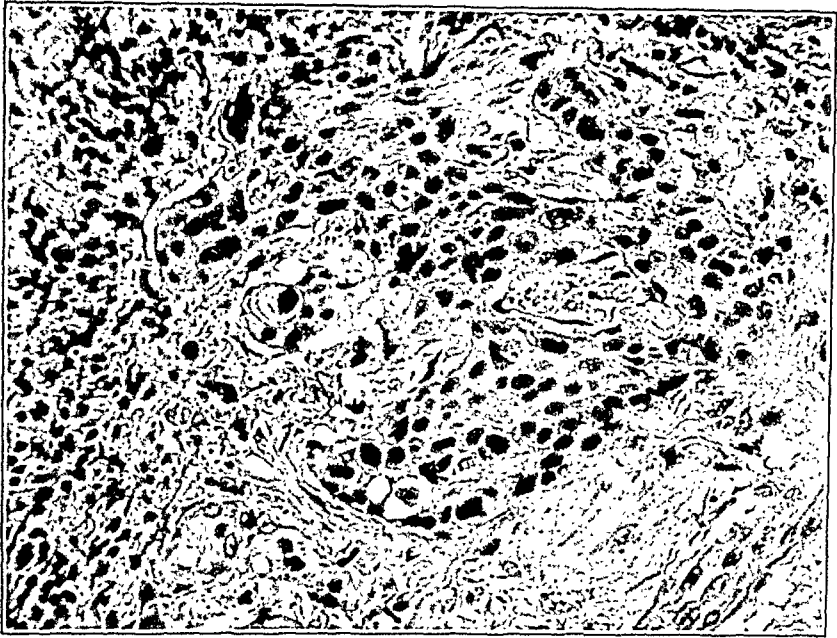
FIG. 2. Primary tumor. One of the cancer plugs is completely necrosed. The connective tissue stroma is active, proliferating and sarcomatoid in nature. In the center a few mitotic figures are seen; one of these shows abnormal mitosis. $\times 600$.

FIG. 3. Lymph node. The lymphoid tissue is replaced by a tissue resembling the sarcomatoid stroma surrounding the necrotic cancer plug in Fig. 2.

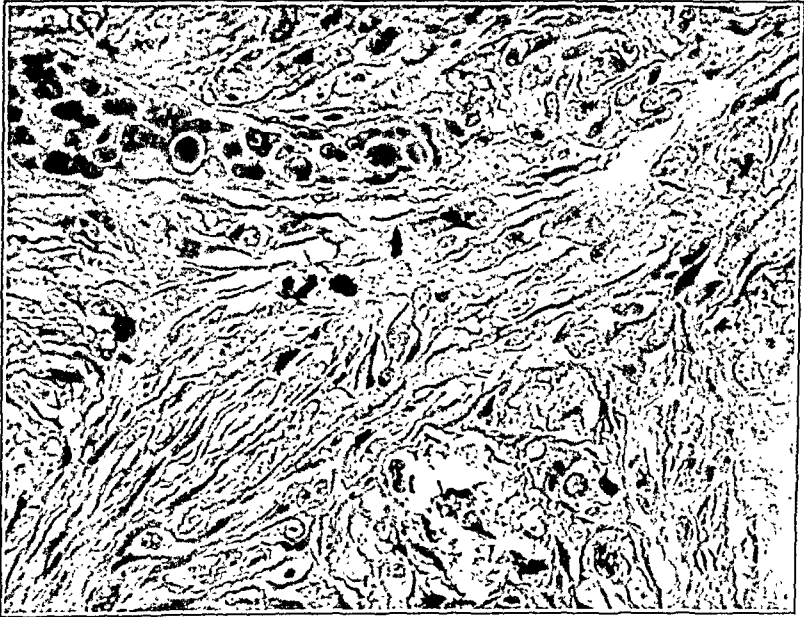
At left, spindle-shaped cells cut longitudinally.

On the right, most of the cells cut transversely. Lower right, a few mitotic figures. Upper right, a large hyperchromatic nucleus is seen. The fine intercellular stroma and collagenous fibrils are visible. $\times 600$.

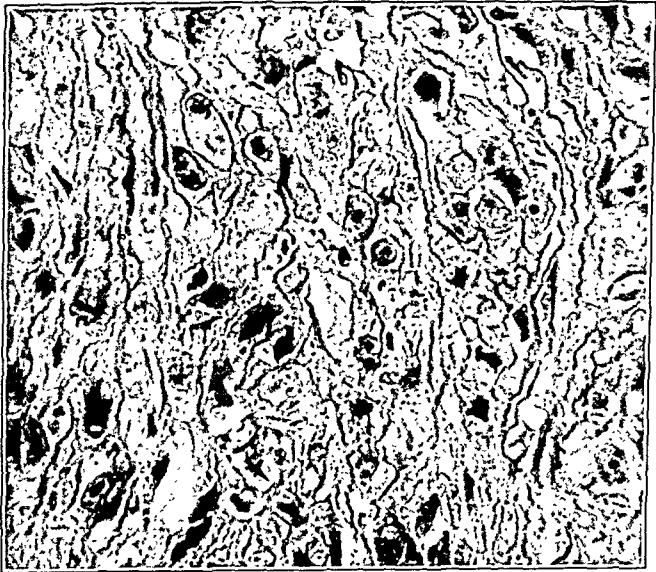
Masson's hemalum-erythrosine-saffron stain is the routine stain used in our laboratory.



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POSTERIOR PITUITARY ACTIVITY FROM AN ANATOMICAL STANDPOINT *

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Since representatives of all departments of medicine are now in pursuit of the manifold activities of the pituitary body it may not be untimely to reconsider the histological evidences of the source and nature more particularly of the posterior lobe secretion and its pathway of discharge.

Because of the great interest aroused by the discovery in extracts of the anterior lobe of separable growth-promoting and sex-maturing hormones, attention of late has been focussed chiefly on the activities of that part of the gland. The posterior lobe meanwhile has been largely ignored and the impression has gained ground that it is a relatively unimportant structure. It has been separately extirpated in dogs without recognizable consequences (doubtless because it was not known what effects to look for), and the recently aroused interest in the hypothalamus and its functions has served more than ever to throw it into the shade.

Herring's Hyaline Bodies: After Howell's demonstration (1898) that the previously discovered haemodynamic effects of pituitary extracts were a property of the posterior lobe alone, ten years elapsed before the publication of P. T. Herring's illuminating studies^{9,10} of this particular subdivision of the gland. He described, in the pars nervosa of cats, what he called hyaline bodies,[†] which were looked upon as the secretory product of the pars intermedia.

* Received for publication June 9, 1933.

† It was possibly an unfortunate designation as the term "hyaline" suggests to pathologists a form of tissue degeneration. Others speak of the same substance as "colloid."

These bodies or masses made their way through the nervous tissue, along what were taken to be lymph spaces, in the direction of the infundibular cavity into which some of them appeared to pass.

This conception of posterior lobe secretion, though accepted wholly or in part by a few observers (*e.g.*, Cushing and Goetsch,⁷ da Costa,⁵ Remy Collin,⁴ Biedl³), has been looked upon in most quarters as wholly fantastic. Attempts on the part of many to establish the actual presence of posterior lobe hormone in the cerebrospinal fluid have led to contradictory results. Possibly the two most convincing recent observations in support of this view have been: (1) by Karplus and Peczenik¹¹ (1930), who found a pressor substance in the cisternal fluid only after electrical stimulation of the tuber; and (2) by Zondek and Krohn²² (1932), who have shown that a melanophore-expanding substance (*Intermedin*) proves not only to be abundant in extracts of the pars intermedia, of stalk and of tuber, but is also demonstrable in small amounts in the fluid of the third ventricle. In the cerebrospinal fluid elsewhere it is not detectable.

Important information regarding the tubulo-infundibular apparatus has meanwhile been secured from two sources. One of them has been the demonstration (1925), independently by Greving⁸ and by Pines,¹⁴ of the rich network of unmyelinated fibres from supra-optic and tuberal nuclei which arborize in the posterior lobe, thereby confirming Tello's observation²⁰ (1912) on the human gland of what Ramón y Cajal¹⁸ had originally (1894) described in the mouse. The other has been the description by Popa and Fielding^{15, 16, 17} (1930-1933) and Basir² (1932) of an "hypophysio-portal" circulation whose main venous trunks, having gathered blood from the sinusoids of the pars distalis, ascend in the pars tuberalis to break up in a vascular capillary plexus in the tuber cinereum (*cf.* Figs. 2-12). It is assumed that these ascending veins, which were independently described by Pietsch¹³ (1930), serve to carry the products of pars anterior secretion, colloidal masses of which may occasionally be demonstrated¹⁷ in the lumina of the vessels.

There thus appear to be two routes, both probably under nervous control, whereby the products of pituitary secretion are transported to the tuberal and possibly to other nuclei of the anterior hypothalamus: (1) from pars anterior by way of the "hypophysio-portal" vessels; and (2) from pars intermedia by way of the tissue

spaces in the pars nervosa. Though something will be said of the former route of secretion, it is with the latter that this paper will chiefly deal.

Among those who have described the course of the hyaline bodies through the pars nervosa and who believe that it has a stimulatory effect on the tuberal nuclei, only a few (Herring in the cat, Cushing and Goetsch in the dog, and Remy Collin in birds) appear to have noted that hyaline masses may actually be seen squeezing their way between the ependymal cells to enter the infundibular cavity. Observations of this kind are fortuitous because there is no certain method of preserving the secretory product in the tissues. For owing to the ready solubility of the substance it is usually dissolved out in the process of tissue fixation, leaving only an occasional hyalinoid mass still retained in the empty spaces in the open glial meshwork. That these empty tissue spaces which comprise the greater part of pars nervosa, stalk, tuber and infundibular ependyma serve as a tell-tale of the former presence of the secretory product, an effort will be made herein to show.

The Source of the Posterior Lobe Hormone: Preparations of the active principle of the posterior lobe are customarily made from the glands of animals in which there is a residual cleft. Hence, the extracts are not solely of pars nervosa, as generally assumed, but of pars intermedia as well. It is scarcely conceivable that the neural core of the lobe is capable independently of elaborating a hormone, which obviously must be produced by its inseparable epithelial investment. The two are in intimate functional contact, for the infundibular projection represents the only portion of the central nervous system that remains unprotected by an essentially impermeable pial covering.

Because of the absence of a distinct cleft in the glands of man and higher apes certain writers disclaim the existence in them of a separately recognizable pars intermedia. This, however, is an academic point of view that has been well answered by Rasmussen¹⁹ (1930), who believes with others that the elements comprising the pars intermedia are clearly distinguishable. Even under normal conditions, when the lobe is relatively inactive, the chromophobe mother cells of the juxtaneural investment tend to arrange themselves into colloid-holding vesiculi, from which an occasional ripened cell may be seen invading the adjacent neural tissue.

In the process of their ripening the cells of the pars intermedia invariably become transformed into basophilic elements which in their mature stage are indistinguishable from the ripened basophiles of the pars distalis. The cells may occasionally be seen in the process of disgoring their cytoplasm in apocrine fashion, but more often the entire cell body is cast off in the manner of a holocrine secretion (*cf.* Fig. 14). The highly vacuolated elements thus discharged soon lose their tinctorial affinity and each separate mass as it becomes "hyalinized" in its passage into the lobe may long retain the recognizable ghost of the swollen nucleus (*cf.* Figs. 15-16). These so-called hyaline bodies of Herring are certainly not the degenerated end-bulbs of nerves as some writers, under the influence of Tello's paper, have come to believe.

Basophilic Activation of Posterior Lobe: When the normal activity of the pars intermedia is exaggerated in certain pathological states there occurs a marked hyperplasia of the basophilic elements. Not only do they discharge their secretory product into the relics of the original cleft, which can be partly reestablished thereby, but the ripened elements wander deeply into the neural tissue after the manner of a malignant epithelial invasion, to which the process first described by Thom²¹ (1901) has often been likened. While this infiltrative tendency has been observed by many, it has been commonly supposed to be a peculiarity of the glands of aged persons (more particularly of those victimized by arteriosclerosis and renal disease) and to have no functional significance.

These infiltrating elements, which are apparently unrelated to the tubulo-racemose glands recently redescribed by Lewis and Lee¹² (1927), are accompanied by a great increase in the hyalinoid secretory product that gives to the lobe, stalk and tuber its "juicy" appearance to the naked eye. And when sections are cut and the pars nervosa is found to be composed of a widely opened mesh (from which the hyalin has been largely dissolved out), it is usually described as representing an "oedematous" condition of the tubero-infundibular structures.

As must have been observed by all who have studied serial sections through this region in whatever plane they happen to have been cut, this familiar "oedematous" or open appearance of the tissue is sharply confined to pars nervosa, stalk, tuber cinereum and neighbourhood of supra-optic and paraventricular nuclei. Attention, however,

does not seem to have been specifically drawn to the peculiar fact that the ependymal cells lining the lower infundibular cavity are invariably broken up by this so-called "oedema," whereas the ependyma of adjacent parts of the ventricle remains more or less intact.

The impression is inescapable from this appearance that something (the secretion in all probability) is forcing the cells apart and being extruded into the ventricle. At the tip of the infundibular cavity there may be no trace whatsoever of the ependymal lining (*cf.* Fig. 17) and patches of it begin to appear only as one passes upward (*cf.* Fig. 18). Not infrequently one sees the more or less intact ependyma lifted into a bleb that has the appearance of rupturing into the ventricle (*cf.* Figs. 19–20), after which a new cuticular layer of cells reforms. Even when the lining of the infundibular cavity is found to be reasonably intact, should the cells happen to be cut lengthwise in the direction of their long tails, the cell bodies here and there seem to be irregularly separated, as though mechanically forced apart. This broken-up appearance of the tubero-infundibular ependyma must have been observed by many and likely enough been ascribed to an artifact from some fault in tissue fixation or section cutting.

Posterior Lobe Basophilia in Disease: Moderate degrees of basophilic infiltration of the pars nervosa have often been described with no suggestion made, or at least no emphasis laid, on the possibility that it represented a physiological activation of the neurohypophysis. And while it is not the purpose of this communication to do more than point out what are looked upon as histological indications of posterior lobe activity, the reasons for bringing the matter up anew at this time may briefly be given.

A long known polyglandular disorder has been shown to be caused by an actively secreting basophilic adenoma of the pars distalis. In the study by serial sections of the pituitary body and interbrain of a fatal case of this disorder (as told in another place⁶), a most extensive infiltration of basophilic elements from the pars intermedia was disclosed. Since the clinical disorder, among other symptoms, is characterized by hypertension, glycosuria and adiposity, which suggest a posterior rather than anterior lobe effect, it was assumed that the cellular invasion of the pars nervosa might well have been the causative factor in these symptoms and might therefore represent a physiological hyperactivation of the posterior lobe.

Aware that Anselmino and his co-workers¹ had found in the blood of patients suffering from the hypertensive toxæmias of pregnancy (eclampsia) a substance giving the reactions of posterior lobe extracts, also that Irvine Page (personal communication) had found a similar or the same substance in the blood of certain patients with so-called essential hypertension, as opportunity has arisen studies have been made of the glands secured from a few fatal examples of these two disorders.

These glands have shown, in somewhat less degree, the same massive basophilia of the pars nervosa seen in the aforementioned polyglandular disorder. It is assumed, therefore, that the process in all probability *does* indicate an hyperactivation of the posterior lobe and consequently represents the pathological basis of these hypertensive maladies. It is perhaps not overventuresome to predict that in some of these hypertensive states the posterior lobe principle may prove to be detectable in the fluid of the third ventricle as well as in the blood stream.



FIG. 1. The original block of tissue (natural size).

Illustrative Material: The accompanying photograph (Fig. 1) shows the original block of tissue from a fatal case of essential hypertension.* It is viewed from behind and shows the small, somewhat cupped, gland with prominent posterior lobe, the stalk and tuber being distinctly "juicy" in appearance. After fixation in formalin the entire block was cut serially from below upward on the horizontal plane in 8 micron sections, every tenth section having been stained with haematoxylin and eosin† and numbered accordingly.

* For this particular specimen I am indebted to Dr. George Hass of the pathological department of the Peter Bent Brigham Hospital.

† The preferential method (1) of fixing, (2) of cutting and (3) of staining the pituitary body naturally differs with what may be the object of the study. Da Costa,⁵ for example, states that the hyaline bodies are better preserved in small tissue fragments fixed in Flemming's solution, with which we have had no experience. For our particular purposes it is undesirable that the gland should be cut in parts before fixation, as is commonly done. Hence, owing to its greater penetrability, formalin has distinct advantages over Regaud's, Zenker's or Kolatchew's fluid.

For our purposes also, serial sections in the horizontal plane prove to be preferable to coronal or sagittal sections as they give better topographical orientation, particularly should the block include the tuberal region as well. (Continued on opposite page.)

Photomicrographs of certain selected sections from this single case will suffice, it is hoped, to bring out the principal points relating to posterior lobe activity, which it is the purpose of this communication to reemphasize. For orientation all sections should be regarded as being seen from above, the anterior structures lying at the top of the print, the posterior structures at the bottom. Figures 2 to 12 have been chosen for the purpose of tracing on these horizontal sections the "hypophysio-portal" circulation from pars anterior to tuber cinereum. Figures 13 to 20 serve to show the formation (from the inwandering basophilic elements of the pars intermedia) of the secretory product and its upward passage through pars nervosa and stalk toward the ventricle. The legends facing these illustrations (to be found at the end of the text) will render it superfluous to redescribe them here.

SUMMARY

In all the recent attention paid to the pituitary body and its functions, the posterior lobe has been much neglected because of the greater temporary interest in the hormones of the pars anterior and the newly discovered activities of the interbrain. It nevertheless has an active principle or principles capable among other properties (1) of raising blood pressure, (2) of contracting smooth muscle, (3) of causing hyperglycaemia, (4) of expanding cutaneous melanophores, and (5) of diminishing renal secretion.

Its secretory product is unmistakably derived from the investing pars intermedia, whose cells become basophilic when ripened. Doubtless under nervous impulses from hypothalamic nuclei these cells, by a form of holocrine secretion, are cast off, invade the pars nervosa and become transformed into "hyaline bodies," which as Herring first showed in lower animals make their way through the loose neural spaces of pars nervosa in the direction of the infundibular cavity. They may, in favourable preparations from the cat and

Simple haematoxylin and eosin stains give perfectly satisfactory cellular differentiation when the solutions are properly used. While many stains, like Mann's eosin-methyl blue and Mallory's aniline-blue orange G, give a more brilliant colour contrast, this is often at the expense of a clear picture of the cellular morphology. The specific stains for the granules, such as Bailey's acid-violet eosin or ethyl-violet orange G, after Regaud fixation, or Severinghaus' acid-fuchsin methyl-green acid-violet stain after Kolatchew fixation, for the reasons given are inapplicable to the serial study of a large block of tissue.

dog, even be seen to extrude themselves into the ventricle between the ependymal cells.

In certain conditions of disease characterized notably by hypertension, but also by other symptoms suggestive of known physiological effects of posterior lobe extracts, the normal cellular activity of the pars intermedia becomes greatly exaggerated. This is shown by a marked hyperplasia of the basophilic elements that penetrate far into the lobe. Under these circumstances not only is the visible secretory product (hyalin) greatly increased in amount over what is customarily seen in supposedly normal glands, but the cuticular ependyma of the lower third ventricle has a highly broken up appearance.

An extreme degree of cellular hyperactivation of the posterior lobe was first observed in the pituitary body of a fatal case of what is known as pituitary basophilism associated with a basophilic adenoma of the pars distalis. Since then a similar condition has also been found in cases of eclampsia and of so-called essential hypertension. In both of these latter states there has been found in the blood stream a substance indistinguishable in its effects from posterior lobe extract, but not detectable in the blood under normal conditions.

The conclusion is drawn that the infiltrative basophilia of the pars nervosa is an expression of functional hyperactivation of the posterior lobe and may be taken to represent the pathological basis of these hypertensive disorders. What particular form of neurohumoral stimulus primarily incites the neurohypophysial basophilia remains to be determined.

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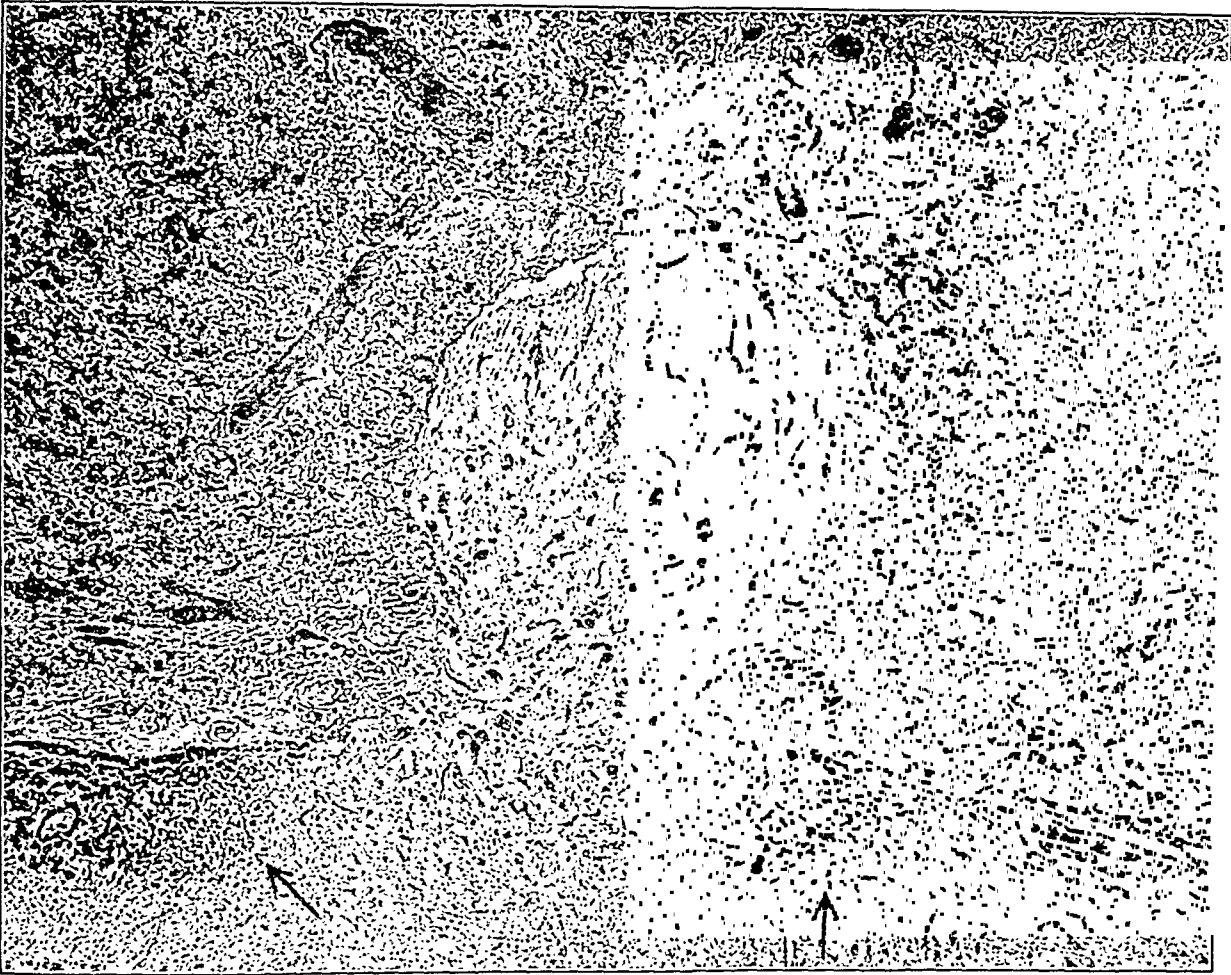
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DESCRIPTION OF PLATES

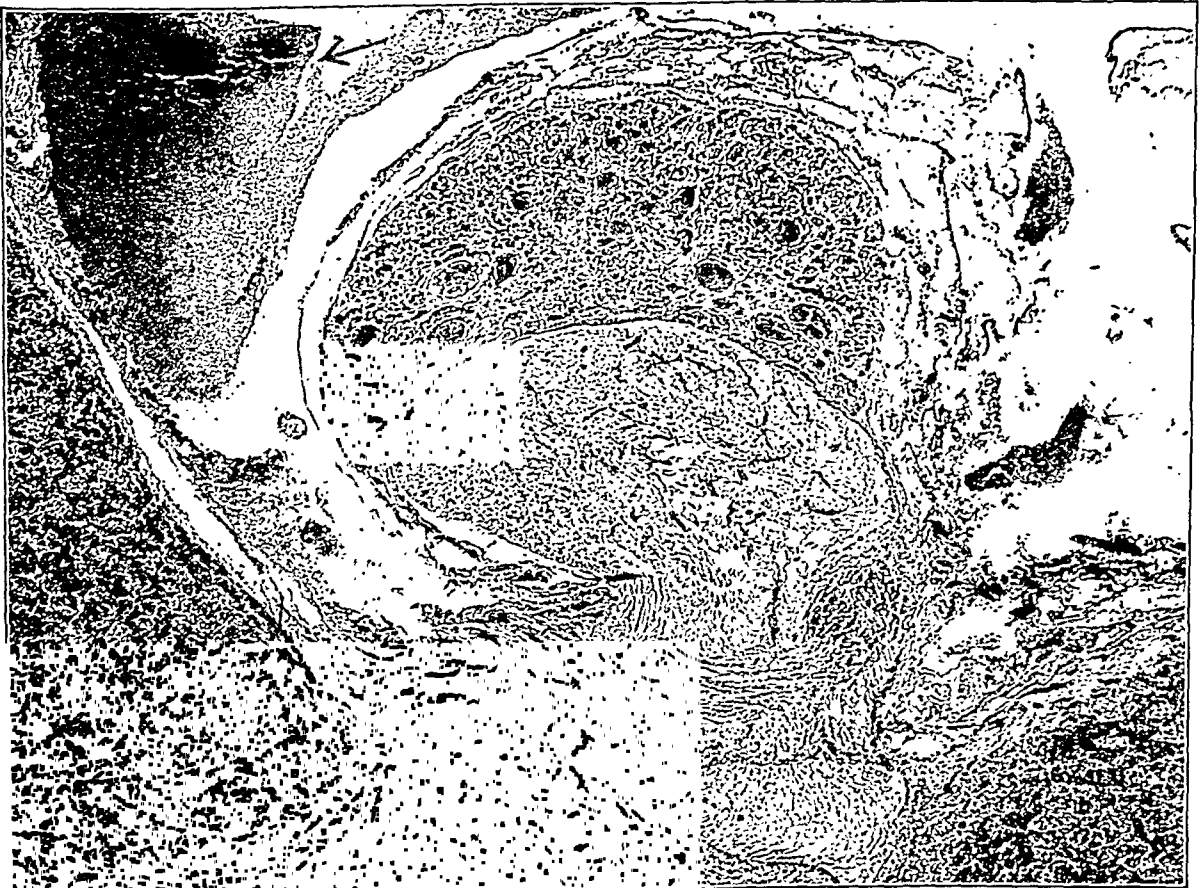
PLATE 75

FIG. 2. (Section No. 40: mag. $\times 30$.) This section transects the upper part of the gland where chiefly acidophilic elements abound. Three large collecting sinusoids can be seen in the pars anterior (above) passing in the general direction of the root of the stalk which appears in the centre of the field. Even at this high level the infiltration of the pars nervosa by basophilic elements (arrows) from pars intermedia is abundant (*cf.* Fig. 13).

FIG. 3. (Section No. 52: mag. $\times 30$.) This section passes through the base of the emerging free stalk and shows the anteriorly placed situation of the pars tuberalis through which pass the large ascending "hypophysio-portal" venous trunks. At the upper left (arrow) lies a small subarachnoid clot from an agonal extravasation. Numerous, minute, capillary haemorrhages are present throughout the pars nervosa, not visible at this low magnification.



2



3

Cushing

Posterior Pituitary Activity

PLATE 76

FIG. 4. (Section No. 61: mag. $\times 30$.) At this level the stalk is entirely free and beginning to be completely enveloped by the cuff of pars tuberalis. This still holds the many ascending portal trunks, which, as Popa and Fielding have shown, are without cross anastomoses.

FIG. 5. (Section No. 85: mag. $\times 30$.) This shows the tendency for the cuff of pars tuberalis to swing toward the sides and back of the stalk as the tuberal enlargement is approached.

FIG. 6. (Section No. 115: mag. $\times 30$.) Here the stalk is enlarging into the tuber at the near proximity to the chiasm shown in the upper part of the field. The "hypophysio-portal" vessels are beginning to leave the cuff of pars tuberalis to plunge into the nervous tissue, forming glomerulus-like tufts (the "secondary capillary net" of Popa and Fielding) enveloped by comparatively dense glial sheaths (*cf.* Fig. 12).



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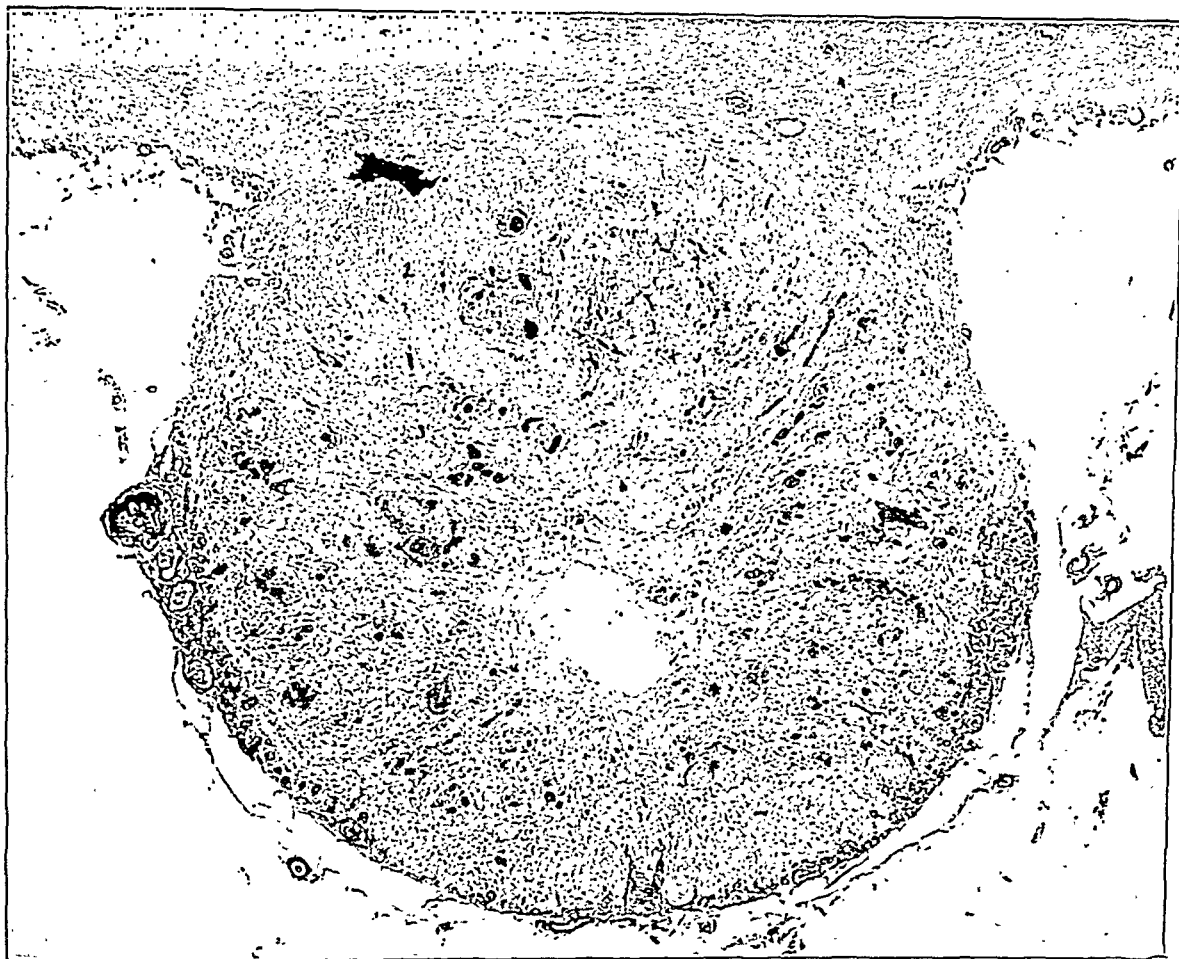
Cushing

Posterior Pituitary Activity

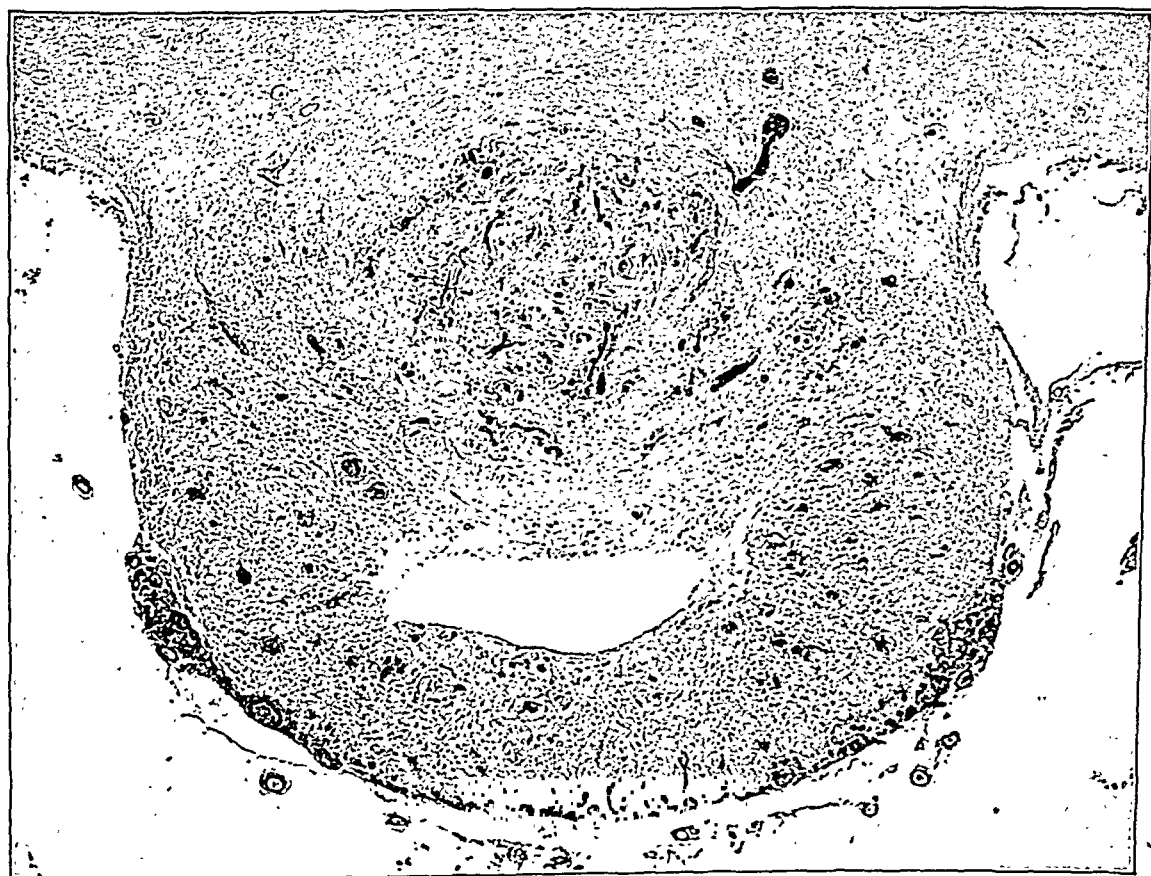
PLATE 77

FIG. 7. (Section No. 124: mag. $\times 30$.) Here the tuber cinereum is fused with the posterior margin of the chiasm, narrow residuals of the pars tuberalis being still evident on its posterior and lateral surface. The tip of the infundibular cavity with its disrupted ependyma (*cf.* Fig. 17) is apparent in the lower centre of the field.

FIG. 8. (Section No. 133: mag. $\times 30$.) The terminal capillary bed of "hypophysio-portal" vessels has here largely swung around to lie anterior to the infundibular cavity. The cuticular ependyma of the ventricle is still missing on the anterior side, but posteriorly the cavity begins to show an intact ependymal lining. Traces of the tuberal cuff are still discernible on the posterolateral surface of the tuber.



7



8

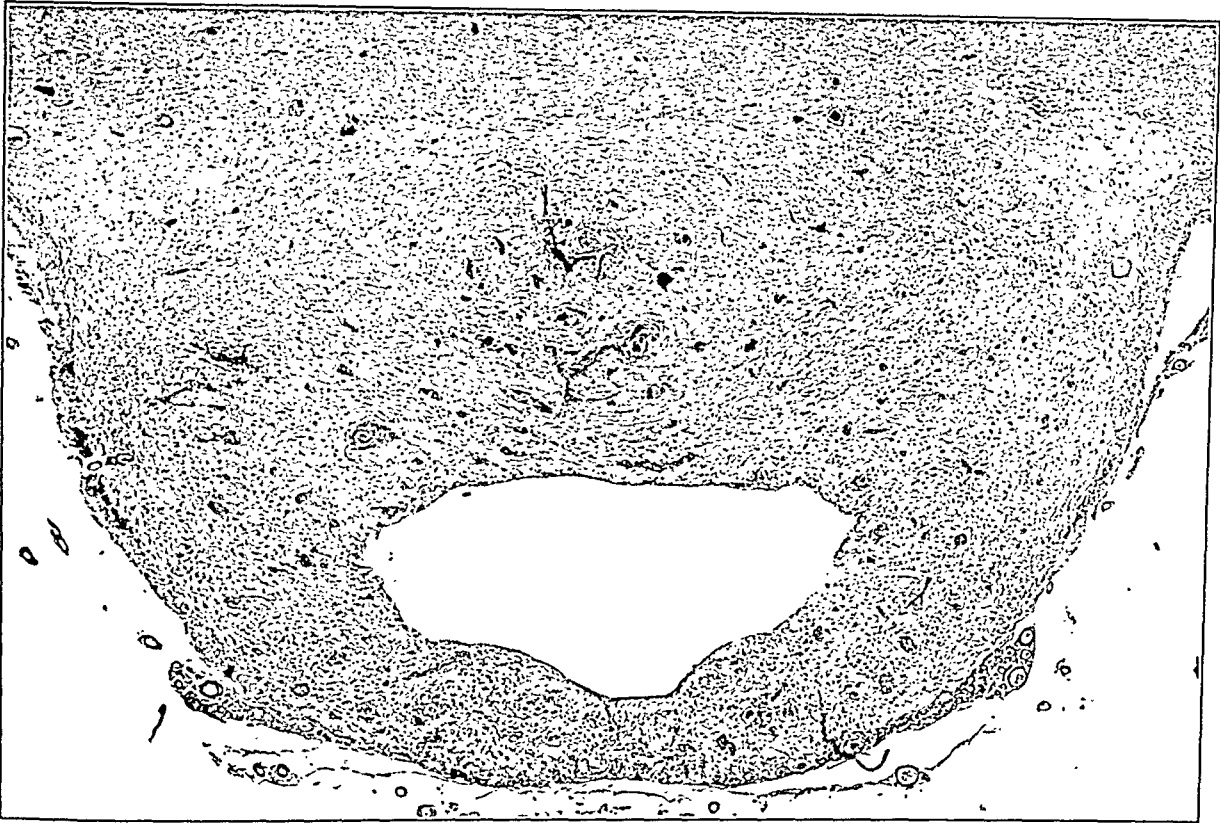
Cushing

Posterior Pituitary Activity

PLATE 78

FIG. 9. (Section No. 142: mag. $\times 30$.) The tuber cinereum here is flattened out against the posterior surface of the chiasm. The loose texture of the tuberal tissue is clearly distinguished by its light shade even at this low power. The cuticular ependyma of the widening cavity is fairly intact, except at its lateral angles. Traces of the terminal capillary plexus of the portal system surrounded by its glial sleeve are still to be seen between ventricle and chiasm.

FIG. 10. Showing (mag. $\times 175$) homogeneous and refractile masses of colloid (?) in two of the ascending hypophysio-portal vessels of upper stalk (arrows). From a case of "pituitary basophilism" due to an adenoma. Colloidal masses in this case are also to be seen in the capillaries of the pars nervosa.



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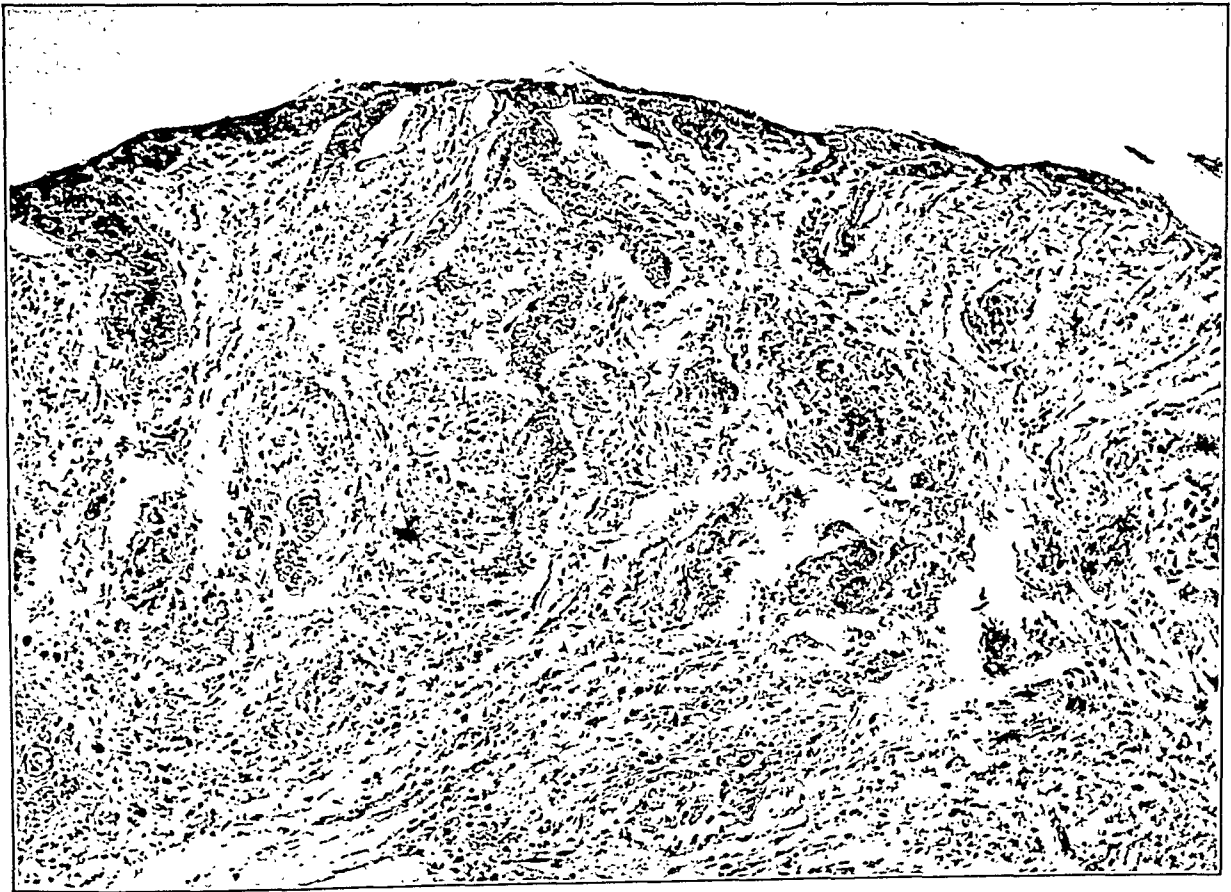
Cushing

Posterior Pituitary Activity

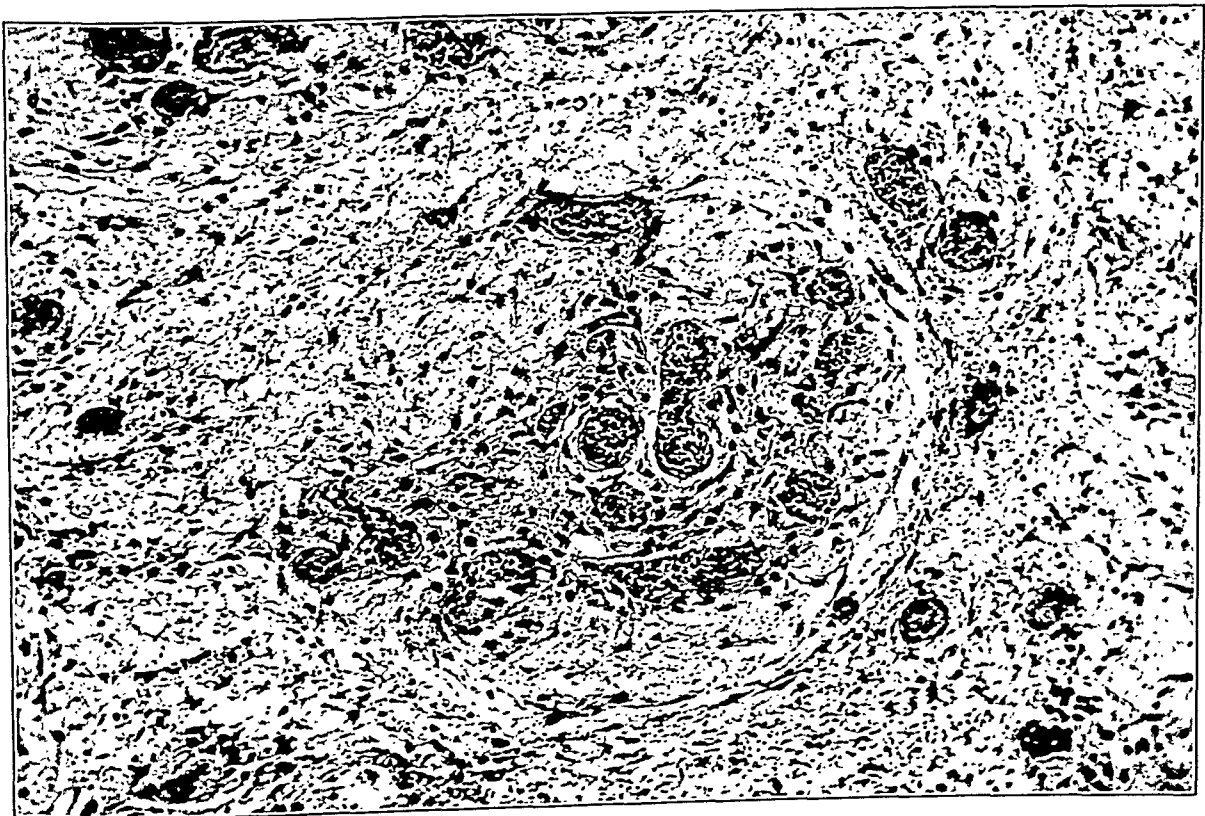
PLATE 79

FIG. 11. Showing (mag. $\times 100$) at level of tuberal enlargement of upper stalk how the large ascending portal trunks break up into smaller vessels which leave the residual of the pars tuberalis to plunge into the tuberal tissue forming the secondary capillary net.

FIG. 12. (Section No. 119: mag. $\times 150$.) A glomerulus-like tuft of capillary vessels with glial sheath characteristic of the vessels composing the secondary net.



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Cushing

Posterior Pituitary Activity

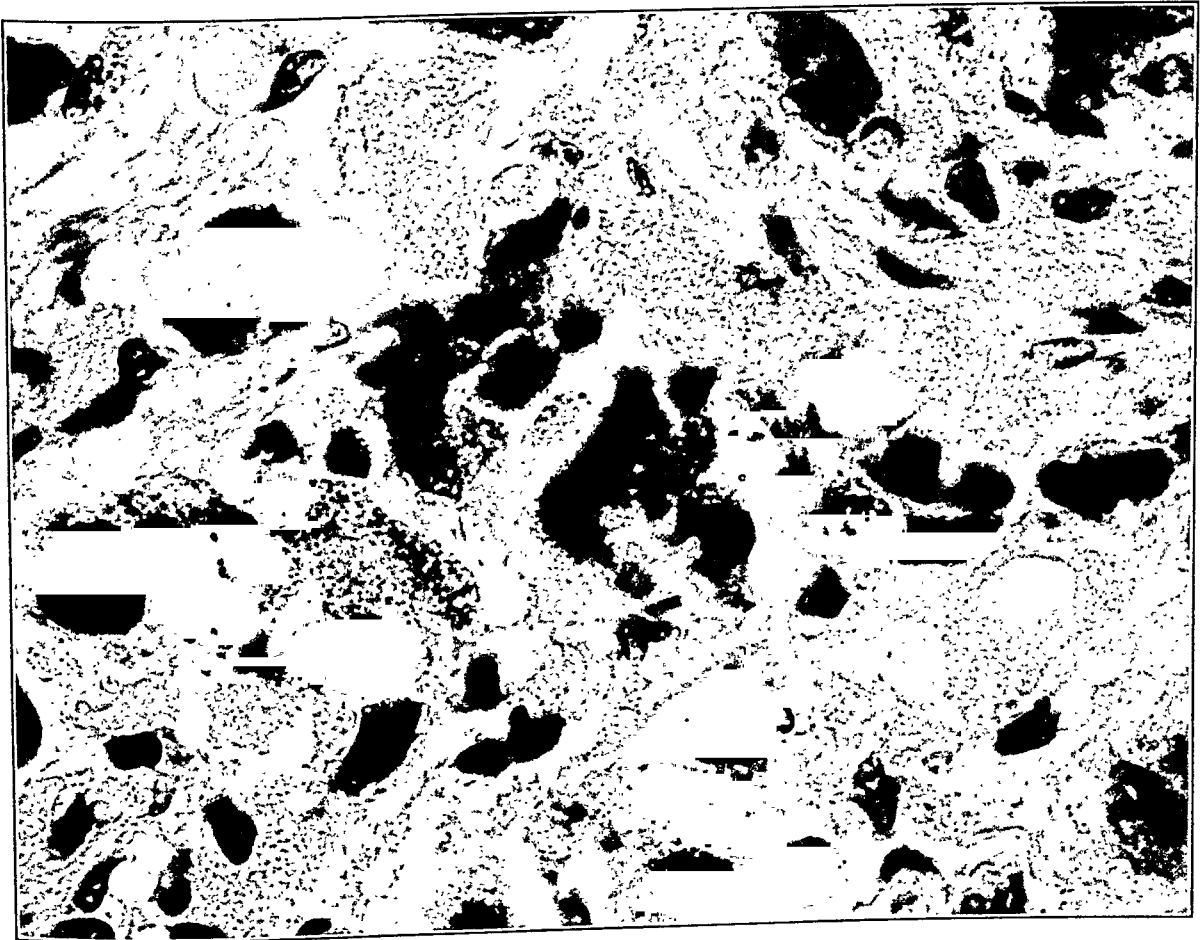
PLATE 80

FIG. 13. (Section No. 34: mag. $\times 80$.) This section passes approximately through the centre of the gland and transects the body of the posterior lobe. It shows the character of the basophilic invasion of the pars nervosa by pars intermedia cells. This invasion occurs in varying degrees of depth from almost the entire juxtaneural epithelial surface.

FIG. 14. Showing (mag. $\times 850$) the masses of secretory holocrine product lying between the tongues of invading and fully ripened basophilic elements. The cast-off cells are still highly granular in appearance but have largely lost their tinctorial affinity to haematoxylin (they stain a reddish brown with Mallory's phosphotungstic acid haematoxylin). In the granular protoplasmic mass of many of the discharged cells the trace of the greatly swollen nucleus is often discernible (*cf.* Fig. 15).



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Cushing

Posterior Pituitary Activity

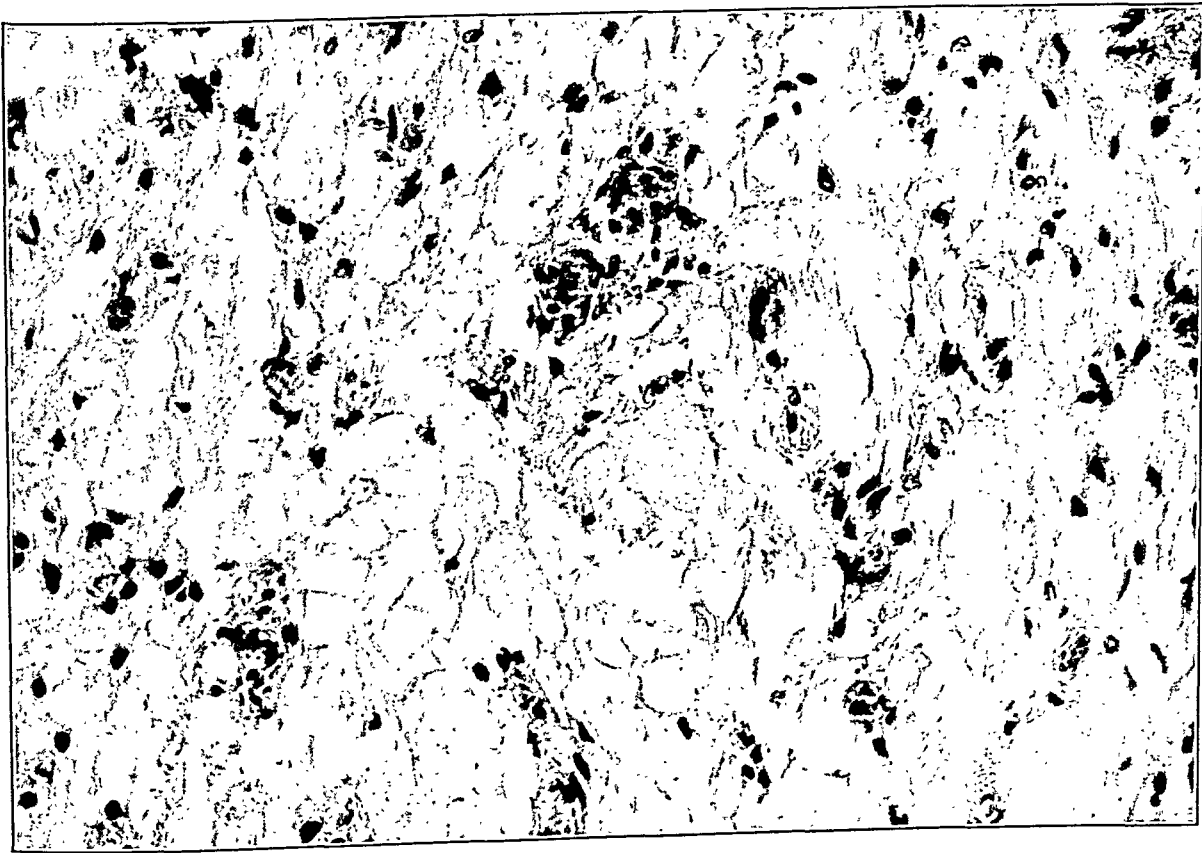
PLATE 81

FIG. 15. To show masses of cast-off holocrine secretion making their way upward through the interstices of the pars nervosa. Ghosts of swollen nuclei still discernible in the secretory masses which become increasingly homogeneous (hyalinized) in their passage through the lobe (mag. $\times 850$).

FIG. 16. Occasional hyaline masses are even to be found trapped in the loose tissue spaces of the tuber. It is assumed from the appearance of the tuberal tissue that the open mesh was once full of the secretory product that has been dissolved out in the process of fixation (mag. $\times 300$).



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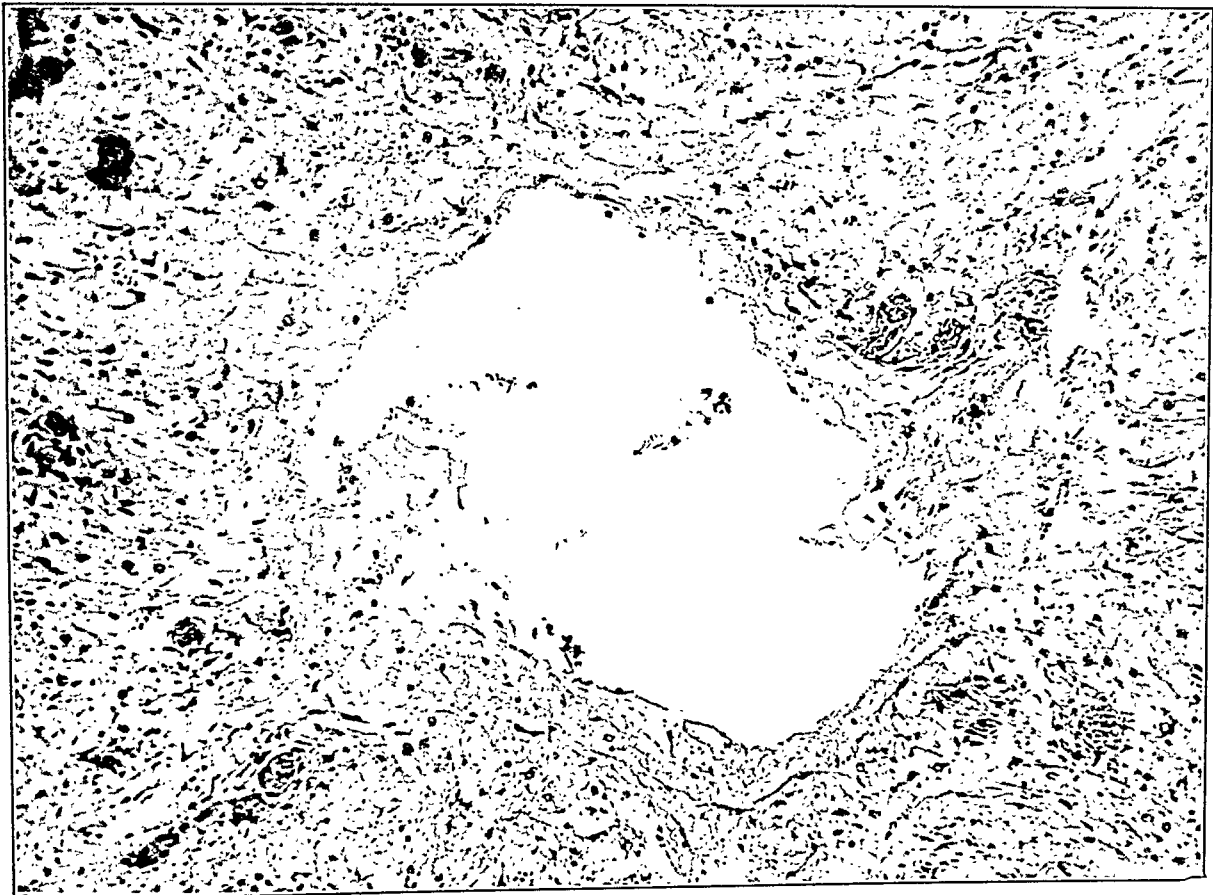
Cushing

Posterior Pituitary Activity

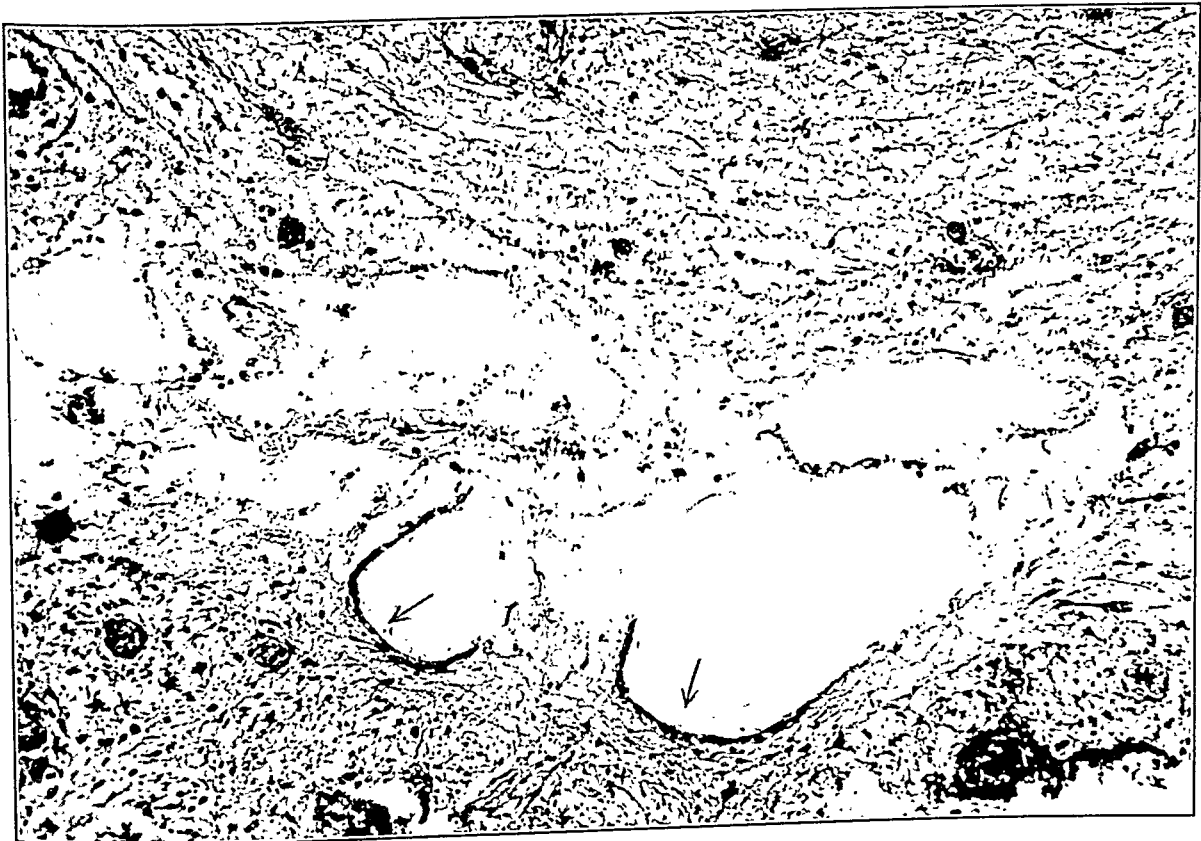
PLATE 82

FIG. 17. (Section No. 124: mag. $\times 125$.) To show at higher magnification a cross-section of the tip of the infundibular cavity (*cf.* Fig. 7). Note the broken-up appearance of the ependymal lining which is invariably present in this region. No cuticular layer is here discernible.

FIG. 18. (Section No. 131: mag. $\times 125$.) To show tip of infundibular cavity slightly higher up than in Fig. 17 (above). Here two loops of intact cuticular ependyma (arrows) are present with an intervening break in the lining. Otherwise one would hardly know that the open spaces represent the ventricular cavity.



17



18

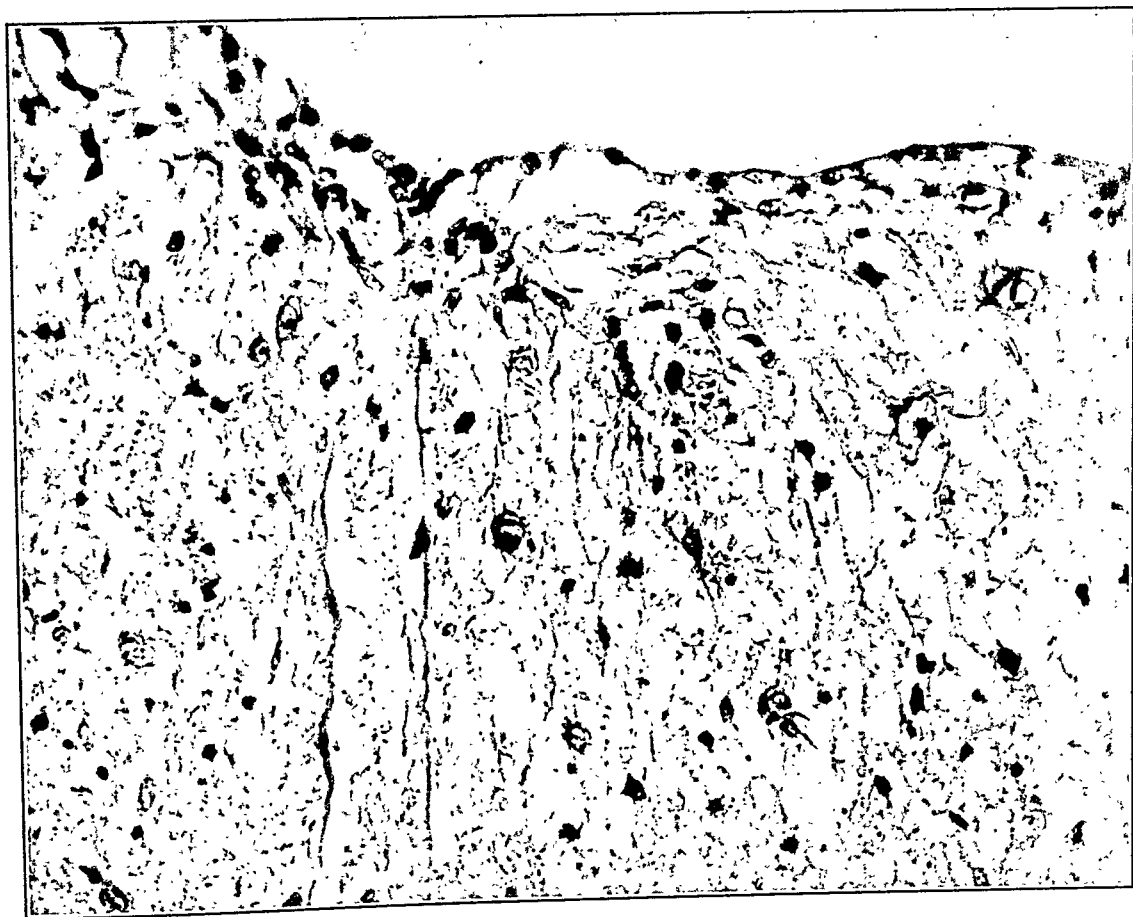
Cushing

Posterior Pituitary Activity

PLATE 83

FIG. 19. (Section No. 151: mag. $\times 300$.) To show a characteristic bleb forming under the still intact cuticle. As these subependymal blebs form, bulge into the ventricle and rupture, a new line of ependymal cells reforms below the protrusion and comes to take the place of the original surface layer.

FIG. 20. A more highly advanced stage (mag. $\times 300$) of what is shown in Fig. 19, indicating the rupture and escape of something (presumably secretion) into the ventricle. Note the tendency of new ependymal bodies to reassemble on the neural side of the bleb.



19



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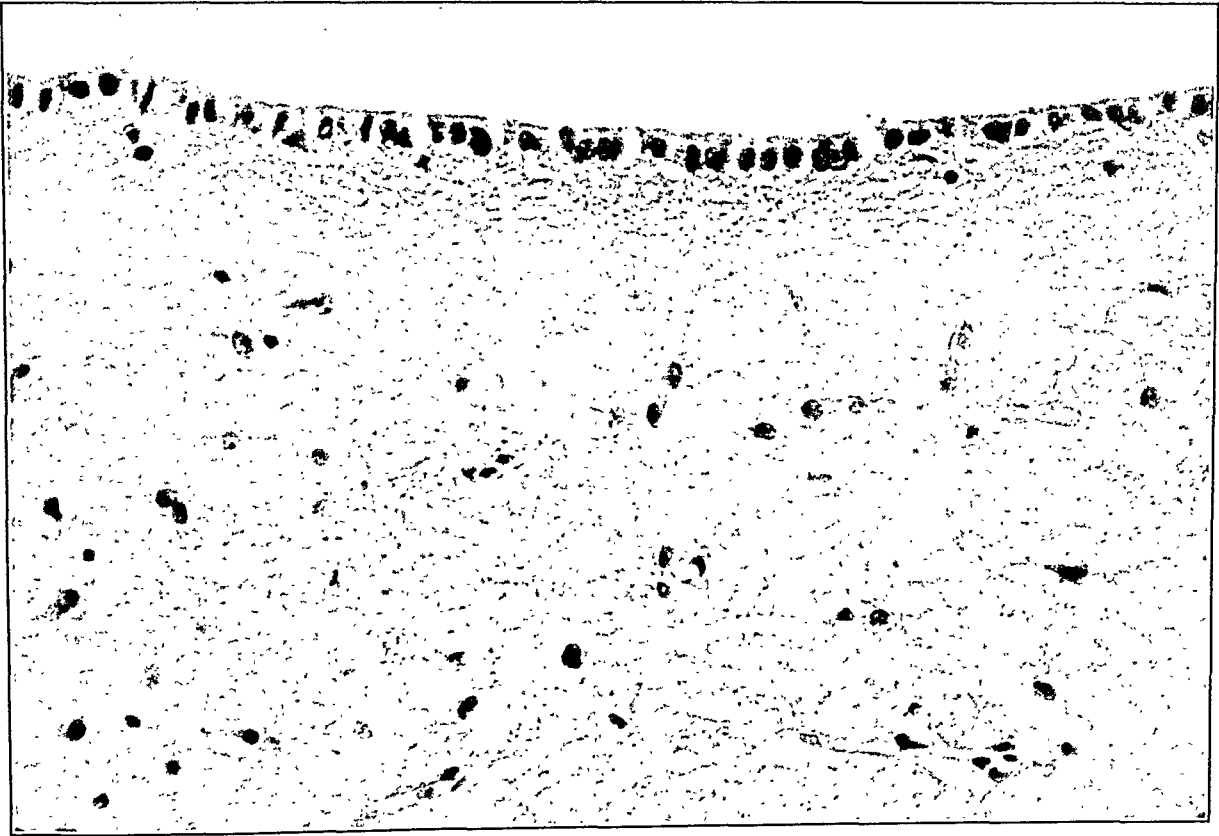
Cushing

Posterior Pituitary Activity

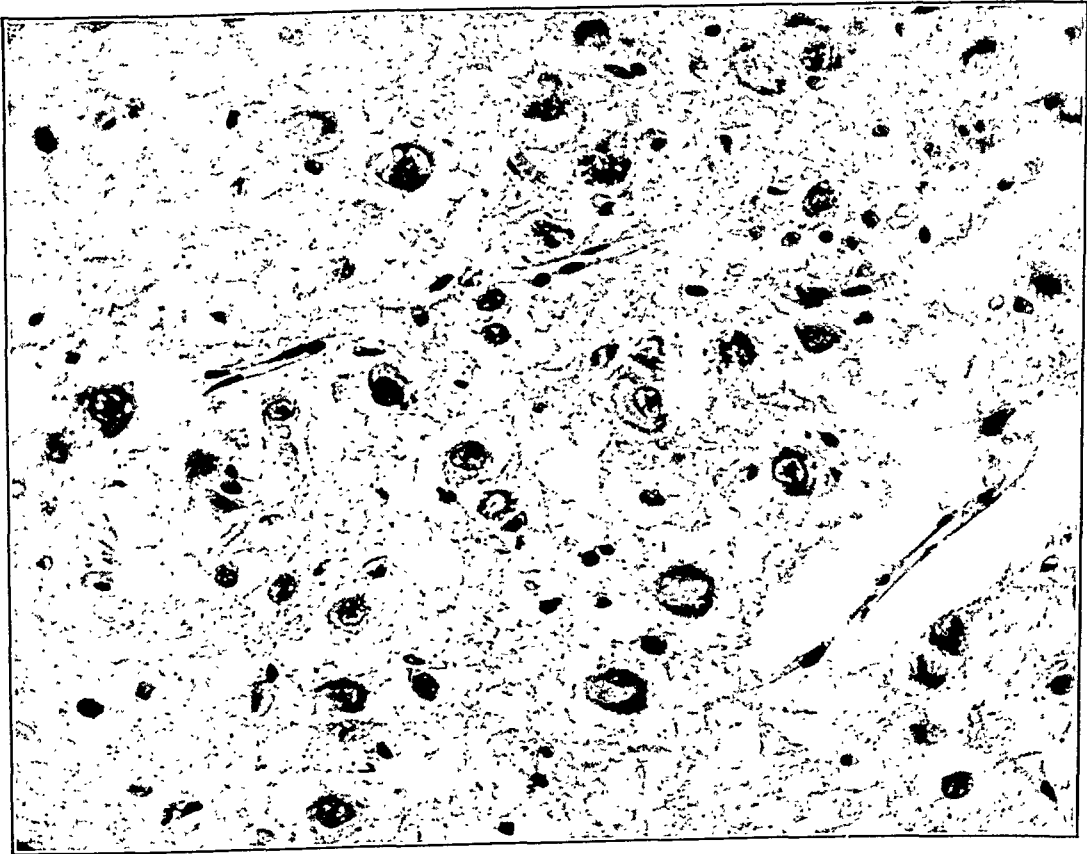
PLATE 84

FIG. 21. In contrast with Figs. 19 and 20, here is shown (mag. \times 300) the fairly intact ependymal lining of the lateral and dorsal aspects of the anterior third ventricle. That this view is taken from the tuberal region is shown by the characteristic open network of the subependymal tissue.

FIG. 22. Here is shown (mag. \times 300) the loose (oedematous) character of the tissue in the region of both paraventricular and supra-optic nuclei, presumably opened out by stimulatory secretion. The adjacent neural tissue elsewhere is normally compact.



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Cushing

Posterior Pituitary Activity

A CASE OF MALFORMATION ASSUMED TO BE TRUE HERMAPHRODITISM *

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Philadelphia, Pa.)*

The subject of the malformation to be described is a middle-aged woman, somewhat slender but of great physical beauty and charm. Her complexion is so fine that she needs no rouge; her hair, which has never been bobbed, is beautiful, long and fine; her bust is of rather more than average size; her voice is a distinct soprano, and she has been happily married to a member of the medical profession for years. At no time has she ever shown any suggestion of any endocrine abnormality. She menstruates regularly every three weeks. In her early married life she was once told by a physician that it was improbable that she would ever have a child, but the patient remembers no reason for this statement and the matter was temporarily forgotten until later when she believed herself to be pregnant. She missed one period, had a little "stomach distress" which lasted a week or two, then, following a typhoid fever inoculation, again menstruated, the clots being a little more numerous and larger than normal. There has never been any other suspicion of pregnancy, although no precautions for its prevention have been taken.

Some years ago, in preparation for an operation for the removal of a urethral caruncle, the external genitalia were examined and no malformations of any kind detected. The clitoris was not enlarged. At that time a routine vaginal examination was made. Something "seemed to be wrong" and the surgeon jumped to the hasty conclusion that a panhysterectomy had been performed sometime previously and the vagina shortened. It was later learned that she had never before been operated upon.

In the early part of 1931 the patient had a fall and shortly afterward discovered a swelling in the groin. As it soon disappeared, it

* For the specimen, data and permission to publish this interesting case, I am indebted to Dr. Jonathan Forman and to Drs. J. F. and Hugh A. Baldwin, to whom I here tender my appreciation of their generosity and extend my thanks.

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was supposed to have resulted from extravasation. Later, however, it reappeared and upon examination was found to be a femoral hernia. At a convenient time an operation for the correction of the abnormality was performed.

Notes made at the time of the operation describe it as follows: "No hernial protrusion was seen or felt, but the finger could be introduced into the femoral canal. The usual incision was made, the sac easily found, freed, pulled down and opened, exposing what was thought to be a sliding hernia. On pulling this down a body whose size and shape resembled an ovary was found, detached and removed, after which the sac was freed high up, ligated, and the opening closed with two chromicized catgut sutures in the usual manner."

"Examination of the tissue failed to show anything that could be identified as ovarian tissue. One part suggested possible malignancy."

It was because of this suspicion of malignancy that sections of some of the tissues were sent to me, and because of my report that the case was not one of tumor but of hermaphroditism that the whole matter was placed in my hands for study and publication.

As frequently happens when the importance of a specimen is not at first realized, the tissues removed at the operation were badly mistreated. They had been cut through in various directions and then crowded into a very small container of fixative in which they hardened in positions making it impossible to determine accurately the relation of the parts. It has not even been possible to discover which was the top or bottom of the whole or what its attachments may have been. In an endeavor to find out exactly what the relations had been the following answer was received: "After opening the sac of the femoral hernia the mass appeared and, as originally stated, was at first suspected to be an ovary. It was intraperitoneal, that is, if you call an ovary intraperitoneal, and seemed to be attached by a sort of pedicle to the side of, or just within the femoral canal. It was cut off at that point and the base whipped together. The rest of the operation was completed in the usual manner, removing the sac and obliterating the opening with chromicized catgut. No drawing that I could make would help in explaining its location."

Although at first it seemed impossible to learn much from the examination of the gross material, after repeated careful study of

all the parts and fitting them together as well as I was able, Mr. Erwin F. Faber, artist to the medical laboratories of the University of Pennsylvania, succeeded in making the diagrammatic sketch shown in Figure 1, which represents, at about the natural size, a fair approximation of the original arrangement.

At the extreme upper part of the sketch at *a* is a portion of a broad tubular canal, at first glance not unlike a piece of intestine, about the thickness of the ileum and more or less rugose. Microscopic examination, however, revealed that it was serous membrane, and it is probably the excised part of the hernial sac.

Immediately below and attached to it at *b* is an ill-defined, dark colored, fibrillar tissue mass, the nature of which was very puzzling. Histologically it is composed almost exclusively of unstriated muscular tissue with many blood vessels and many narrow epithelial tubules.

Below it at *c* is a sharply defined ovoid body, to which is loosely attached by serous membrane an elongated tubular structure free at the end toward the left and disappearing in the muscular mass to the right, above and behind. The ovoid body was at first supposed by the surgeons to be a prolapsed ovary, and the tubular structure its Fallopian tube, but when the body was incised it at once became evident that it was not an ovary, and they came to the erroneous conclusion that it was a tumor of some kind. At *e* a tiny pedunculated cyst is attached to the free end of the supposed Fallopian tube, while at *g* a larger cyst appears to spring by a long pedicle from the capsule of the body at *c*.

Careful examination of the body *c* shows it to be covered with serous membrane beneath which is a tough fibrous investiture, and to be made up of a uniform substance of firm consistence and of a cream (?) color.

Upon microscopic examination it at once became clear that the body was neither an ovary nor a tumor but a testis, for it consists entirely of closely approximated epithelial tubules. This being the case, the attached tubular structure would not be an oviduct but might be an epididymis. The little cyst *e* might arise from the organ of Giraldes, and the larger one *g* be the stalked hydatid. Assuming all this to be correct it only remains to point out the homology between the fibromuscular tissue and the spermatic cord to make all of the conclusions consistent.

The confirmation of each of the parts by microscopic examination presented little difficulty.

The Testis: As has been said, this organ is surrounded by serous membrane, the tunica vaginalis, and by a tough fibrous investiture, the tunica albuginea. There is no division into lobules and the usual perfectly regular glandular pattern prevails (Fig. 2). The organ is immature and non-functional. Each tubule consists of a well developed basement membrane, layers of doubtful cells (Sertoli cells?) of semicolumnar form attached to the basement membrane and directed toward the centers of the tubules, whose lumens are vague. It is a question whether the structures should be regarded as genital cords with germinal cells, or as seminiferous tubules. By whatever names they are called they are certainly the homologues of the latter.

The Epididymis: There is neither a distinct head (globus major) nor any definite tail (globus minor) and it is evident that the organ is hypoplastic. The position of the stalked hydatid *g* suggests where the globus major ought to be. Sections of the tissue taken at *d*, shown in Figure 4, show the presence of a spermatic duct with collateral tubules. To determine the histological details more accurately blocks of tissue were cut out between the parallel lines at Figure 1 *d* and *f*, and microscopic sections made. At the center of the broadest part of the organ *d* an epithelial-lined, widely dilated canal is found. It is surrounded by a muscular investiture of varying thickness penetrated by many good sized vessels such as might represent the pampiniform plexus. The dilatation of the canal seems to be caused by the collection of a fluid rich in some colloid that has been condensed by the fixative, and holding to the cellular lining during its transformation has torn most of the epithelium from its basement membrane. The cells seem to be atrophic. They are cuboidal instead of columnar, perhaps because of the distending and compressing effect of the fluid contents, and there may have been more than one layer. For the most part the cells are small and the cytoplasm takes more or less of the hematoxylin color, as though atrophic and alkaline.

The muscle is unstriated, occurs in loose fasciculi, is not uniform in thickness, and there is some adipose tissue beneath the tunica vaginalis.

Scattered about in the muscle tissue are numerous tiny canals

lined with cuboidal epithelium. Two sections of one of them are shown in the center of the little square (outlined in black) toward the corner of Figure 4, and are again shown highly magnified in Figure 5. These are regarded as vasa efferentia. Their position is not according to the normal anatomical plan, but it must be remembered that the structures are not only anomalous but also hypoplastic, so that deviations from the normal are to be expected.

Sections cut between the parallel lines at Figure 1 *h* were made in the expectation that they would pass through the seminal duct nearer the globus major. The duct was easily found and presented itself as shown in Figure 6. At this altitude it is lined with low columnar cells without cilia, is notably smaller in diameter than in Figure 4, and contains very little fluid. Again numerous tiny efferent ducts are distributed about, but the globus major is not found in the section.

A fragment of tissue was excised from the back of the mass of tissue Figure 1 *b*, referred to as muscular, at a position immediately behind the inner ends of the parallel lines at Figure 1 *h*, and a puzzling confusion of muscular bundles and epithelial tubules encountered. So difficult was it to account for the appearances that some friends, excellent pathologists, to whom the microscopic sections were shown and who at once agreed with all of the statements and conclusions thus far made and arrived at, felt it necessary to refer this mass to neoplastic growth. But this proved to be an error following the assumption that the larger end of the epididymis, that toward *e* (Fig. 1) was the globus major, or head of the organ, and the more tenuous part toward *g* (Fig. 1) the globus minor or tail that disappears in the muscular tissue to become the spermatic cord. As soon as the reverse of this conception is entertained and the globus major sought near where it should be, that is, in the neighborhood of the stalked hydatid *g* (Fig. 1) it becomes possible to understand that in the case of an imperfectly developed series of organs, one of which, the globus major of the epididymis, is formed by the confluence of many small tubules combining to form a single large one, considerable confusion of structure is not only possible but probable. With this thought in mind the original bewilderment fades away and the structure resolves itself into a mere modification of the duct system.

It is unfortunate that the gross material could not be dissected so that the course of the vas and spermatic cord could be traced. The described conditions of incision and fixation made this impossible.

The numerous sections of the testis studied revealed nothing corresponding to its mediastinum. Those from the cut surface *c* (Fig. 1) were uniformly tubular as shown in Figures 2 and 3, but those from the surface descending between the inner ends of the parallel lines at *h* (Fig. 1) show a tubular structure broken into smaller groups as the supposed neighborhood of the globus major is reached. The tunics seem to lose themselves by breaking into bands that confusedly enter the muscular tissue, and the tubular structure breaks up into groups of still convoluted tubules passing away from the testis proper. Apparently this is the region of the rete testis and vasa efferentia, though these structures are too inadequately developed to be definitely identified. One section, shown in Figure 8, appears much like the rete testis and may be its homologue. It may, therefore, be concluded that no globus major, as such, has been formed, though the essentials of its structure are scattered about.

It is also possible that no vas deferens exists except for the duct that occupies the length of the epididymis, the Wolffian ducts perhaps adopting the female course and development. If that be possible, it accounts for the seminal duct appearing to end blindly toward what is supposed to be the position of the globus minor. It also accounts for the absence of any definite spermatic cord, and the peculiarly irregular and distributed character of the muscular tissue of which it should be constituted.

In the shapeless muscular mass, the histology of which is shown in Figure 7, there is a cylindrical formation, *b* (Fig. 1), separated from the rest by a delicate fibrous investiture and at first promising as the homologue of the cord, but without the vas or any other epithelial canal or structure by which the homology can be established. It seems, therefore, improbable that any definite spermatic cord exists.

From these microscopic studies of the tissue little has been gained beyond the identification of a malformed and hypoplastic set of male sex organs, and the microscopic confirmation of the better formed parts as a testis with its epididymis.

DISCUSSION AND CONCLUSIONS

The discovery of a set of male sex organs in an otherwise perfect female can be interpreted only as an indication of true hermaphroditism, but as the subject of the deformity is living, only middle-aged and in good health, there is little probability that a postmortem examination can be made for many years. Under these circumstances it has seemed wise to record the interesting observation that accident has made possible.

The subject seems to be a true hermaphrodite. To consider her a pseudohermaphrodite would be to assume the existence of only one set of sexual organs, which would be those of the male, one-half of which were removed at the operation for hernia and subjected to examination: to consider her a true hermaphrodite would be to predicate the existence of female organs that have not been seen. But the perfect female habitus of the body, the absence of hirsuties, the soprano quality of the voice and above all the regularity of menstruation, leaving out of account the suspicion of the pregnancy and abortion of the early years of married life, are almost indubitable evidence of their existence and function.

No interstitial cells were discovered in any of the sections of the testis, though that does not preclude their presence, as only a very small proportion of the testis was examined and what the remainder might show is unknown. But, if present, they must have been inactive, for the body and its functions all point to the dominance of the endocrine influences of the glands of the opposed sex. It is scarcely conceivable that a male pseudohermaphrodite would have such perfectly formed female external genitalia and such perfectly developed secondary female characteristics as to appear, feel and behave as a female.

The operation at which the specimens were obtained was performed in the early part of 1932, but although the matter of endocrine alteration was carefully inquired into in the correspondence carried on with the surgeons, there is no evidence of any change in the patient, nor is it to be expected when it is considered that the removed male organs seem not to have shown the least potency during the years that they were contained in her body.

DESCRIPTION OF PLATES

PLATE 85

FIG. 1. Semidiagrammatic representation of the removed tissue submitted for examination.

a = Supposed sac of the femoral hernia.

b = Mass of fibromuscular tissue supposed to be the homologue of the spermatic cord (see Fig. 7).

c = The testis (see Figs. 2 and 3).

d = The epididymis, the parallel lines showing the position of the portion excised for sectioning (see Figs. 4 and 5).

e = Cyst supposed to arise from the organ of Giraldes.

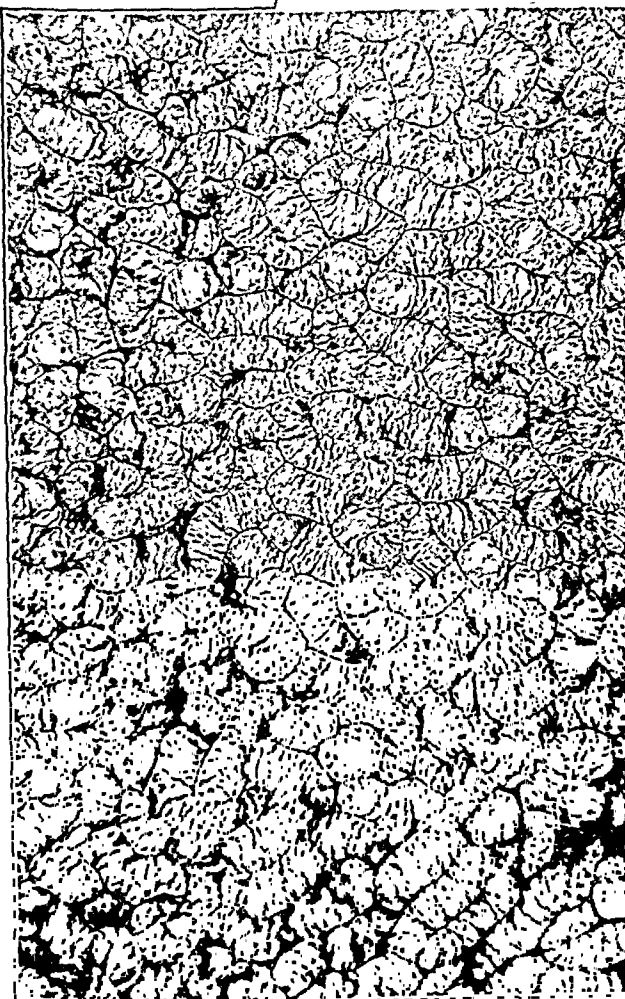
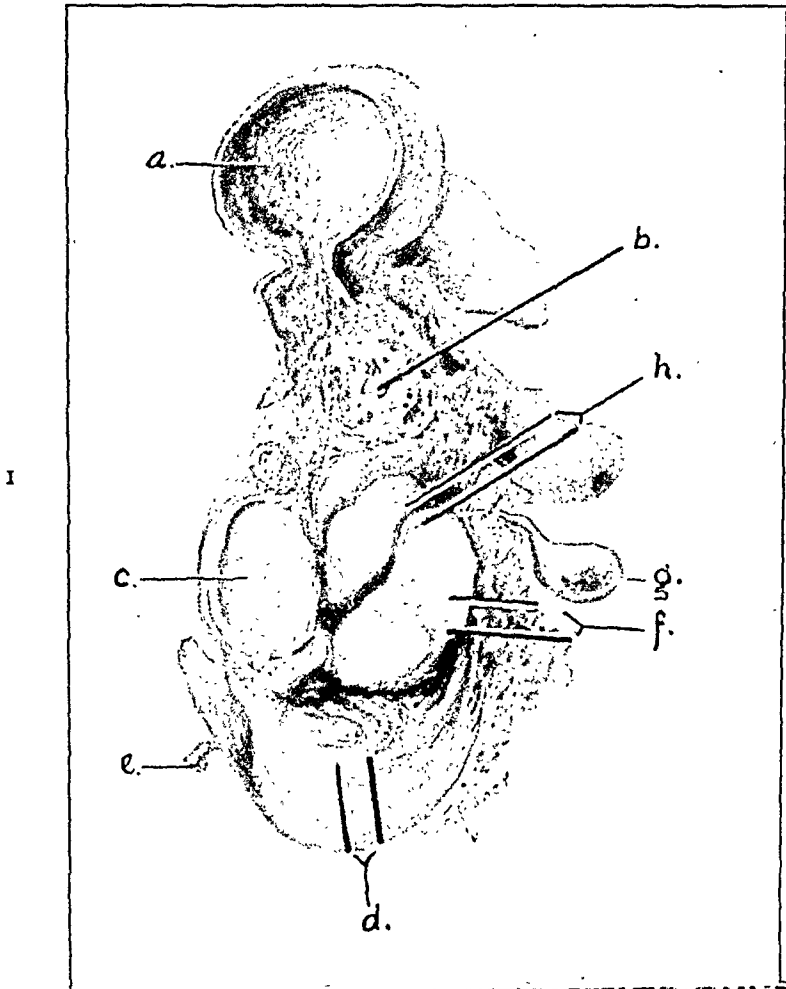
f = Parallel lines showing where another portion of the epididymis was excised for sectioning (see Fig. 6).

g = The stalked hydatid.

h = Parallel lines showing the position of the tissue, section of which proved to be the rete testis (see Fig. 8).

FIG. 2. Showing the perfectly regular tubular structure of the immature testis. See higher power view in Fig. 3.

FIG. 3. Higher power view of the section of the testis showing the tubules, with their well developed basement membranes and somewhat irregular spermatocytes. The immature development has not reached the stage at which primary and secondary spermatocytes, Sertoli cells, and spermatozoa are found.



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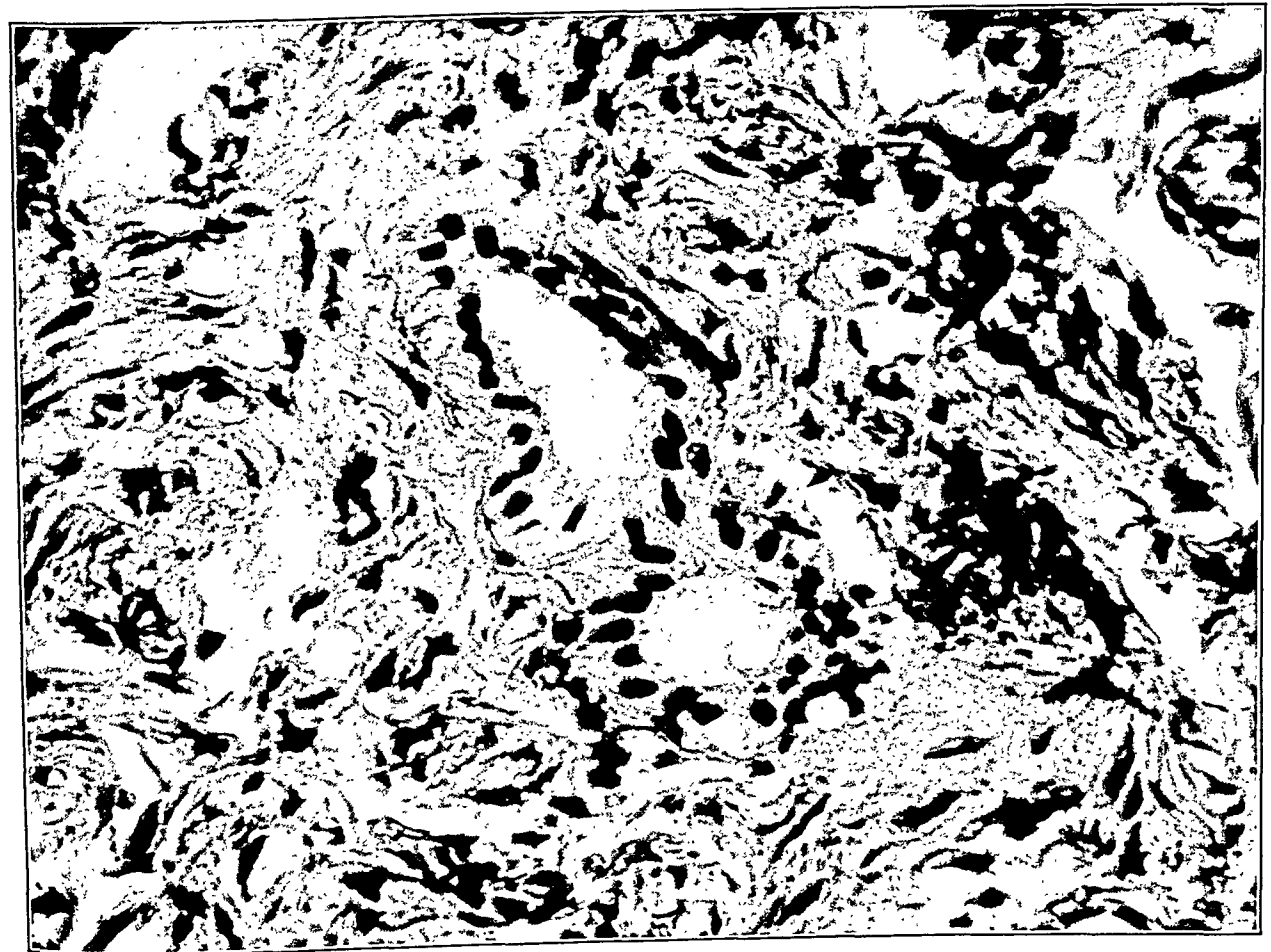
PLATE 86

FIG. 4. Section through the body of the epididymis showing the vas, muscular coats and vessels of the pampiniform plexus, with a section through one of the vasa efferentia in the square. See Fig. 5, a higher magnification of the tissue in the square.

FIG. 5. Higher magnification of the marked square shown in Fig. 4, showing sections of one of the vasa efferentia of the epididymis.



4



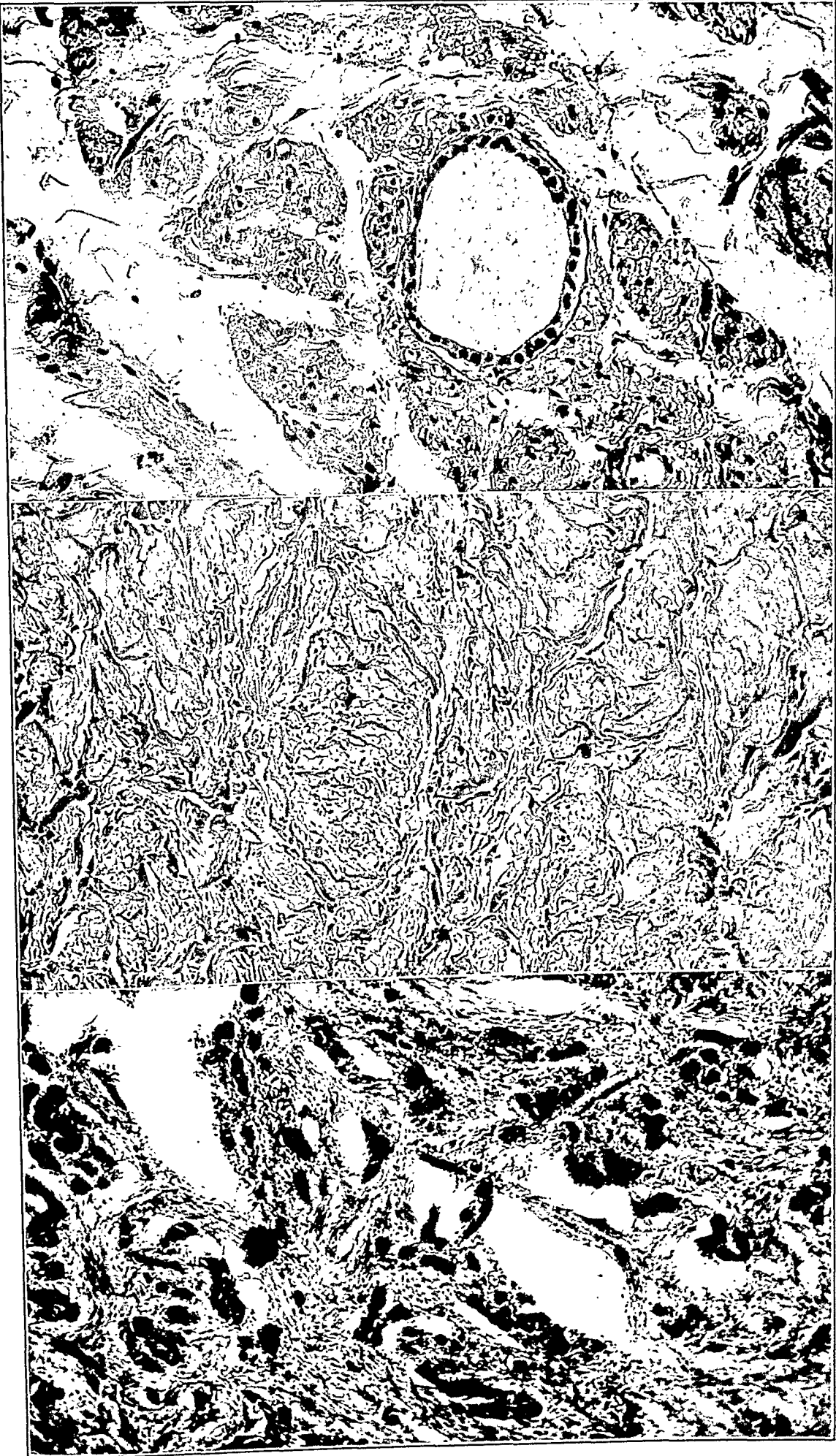
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PLATE 87

FIG. 6. Section through the upper (distal) part of the vas deferens.

FIG. 7. Fibromuscular tissue supposed to be the homologue of the spermatic cord.

FIG. 8. Section through the area supposed to correspond to the rete testis, near the normal origin of the globus major of the epididymis. Bad fixation has caused retraction of the loosely attached epithelium from the spaces it normally lined, and has almost completely closed the lumens.



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PHOSPHORUS AND ALCOHOLIC CIRRHOSIS *

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INTRODUCTION

The object of this paper is to present a certain amount of evidence in favor of the view that so-called alcoholic cirrhosis may be due to chronic poisoning with phosphorus. The idea was first put on record ¹ in the Shattuck Lecture delivered before the Massachusetts Medical Society, June 8, 1932.

The work reported here represents the culmination of thirty-six years' search for the cause of this type of cirrhosis. At an autopsy which I made May 13, 1897 on an adult male there was found a 2350 gm. cirrhotic liver in which microscopically over half of the cells contained in their cytoplasm masses of hyaline acidophilic material (Fig. 1). The lesion in the liver cells was so striking and peculiar that a definite cause for it seemed inevitable. The problem which in time suggested itself was to find that cause.

The search started mildly enough but for the past eighteen years has been an obsession. After ruling out, like other investigators, ethyl alcohol as the cause, every possible toxic substance (over 30 in all) which could be imagined as contaminating liquors was investigated experimentally. The list includes methyl and amyl alcohols, oil of juniper, turpentine, creosote, salicylic and pyroligneous acids, furfural, sulphites, lead, copper, tin, aluminum, antimony and arsenic.

I worked at various times on the problem with three of my former assistants, Drs. McJunkin, Parker and Nye, but of recent years alone. Most attention was paid to lead and arsenic because when given in large amounts they produced acute lesions that seemed promising. This was particularly true of lead, which causes in a very few days the formation of a hyaline basophilic reticulum in liver cells, as reported by McJunkin ² in 1915. Recently I found that a similar lesion was formed at the same time in many of the renal cells. But with small doses given over a long period of time no

* Received for publication June 5, 1933.

comparable pathological changes could be produced, although the attempt to do so was made repeatedly under varying conditions.

During the past five years increasing attention has been paid to phosphorus as the possible cause of so-called "alcoholic cirrhosis," in spite of the fact that no obvious connection between it and liquors was evident. It was tried at first for two reasons: its well known injurious effect on the cytoplasm rather than on the nuclei of liver cells, and the observation that the cytoplasm of the liver cells in a case of phosphorus poisoning contained eosinophilic granules. Experimental work soon showed two strong points in its favor. It readily produces cirrhosis, and when administered in small enough doses over a long enough time gives rise to peculiar degenerative changes in the cytoplasm of the liver cells. The evidence obtained at first was purely morphological but it was promising. In order to appreciate its significance it is necessary to describe first the characteristic and diagnostic lesion of alcoholic cirrhosis.

ALCOHOLIC CIRRHOSIS

The commonest type of cirrhosis of the liver is that known as alcoholic cirrhosis, because it is so frequently associated with over-indulgence in alcoholic beverages. In our series of cases at the Boston City Hospital covering thirty-six years, and all verified by microscopic examination, it forms about 50 per cent, as shown in the accompanying table.

TABLE I

Cases of Cirrhosis at the Boston City Hospital (1897-1932 Inclusive)

Total number of autopsies during the past 36 years ... 10,016

Total number of cases of well marked cirrhosis of

the liver 590 = 5.89 per cent

They are classified as follows:

	No. of Cases	Per Cent
1. Alcoholic cirrhosis	293	49.66
2. Pigment cirrhosis	50	8.47
3. Healed acute yellow atrophy	48	8.13
4. Syphilitic cirrhosis	28	4.74
5. Colon bacillus cirrhosis	27	4.57
6. Obstructive cirrhosis	27	4.57
7. Obstructive and colon bacillus cirrhosis.....	6	1.08
8. Cancer cirrhosis	1	0.16
9. Unclassified	110	18.66

The diagnosis of this variety of cirrhosis is based on the presence of a fine to coarse acidophilic reticulum in the cytoplasm of the

liver cells. With prolonged staining, especially after fixation in alcohol, the reticulum can be stained fairly deeply basophilically both by methylene blue and by alum hematoxylin. The lesion is sometimes found before any evidence of cirrhosis is present and occasionally complicates pigment cirrhosis. In livers in which the cirrhotic process is very active the hyaline reticulum may be present, as in the first case studied, in over half of the cells, but when the process is very chronic only a few affected cells may be found and these are usually old cells at the periphery of the islands of regeneration.

The lesion in the liver cells is, as already stated, both characteristic and diagnostic. Old decolorized bile occurring in liver cells as the result of bile stasis may become acidophilic, but occurs as separate, round, hyaline globules of various sizes (Fig. 4). This type of lesion is sometimes found associated with true hyalin in cases of alcoholic cirrhosis in which focal bile stasis has occurred. The reticulum produced by very acute lead poisoning is basophilic and of very rare occurrence. But one example of it has occurred in my personal experience.

The degenerative changes occurring in the cytoplasm of the liver cells are found throughout the lobule but are usually most marked, at least in early cases, around the central vein. The cirrhotic process on the other hand starts in the portal regions and extends irregularly into the lobule.

Fatty infiltration of the liver cells is a frequent complication of alcoholic cirrhosis and, especially in the earlier stages of the process, often causes marked increase in the size of the organ.

The lesion of alcoholic cirrhosis begins as very fine acidophilic granules in the cytoplasm of the liver cells. They enlarge, coalesce and form a hyaline reticulum that often surrounds the nucleus (Figs. 5 and 6). The reticulum varies according to age and rapidity of formation (Figs. 1, 2 and 3) from fine to coarse, and eventually loses its marked acidophilic property so that it stains lightly or not at all. If the cell dies, the reticulum is attacked by polymorphonuclear leukocytes or monocytes and gradually dissolved. As I have previously stated, I have always regarded the reticulum as the fingerprint of the agent that causes this type of cirrhosis. It is the only clue to its identity that we possess.

Study has shown that the acidophilic granules and reticulum have

certain physical and chemical properties. They stain deeply with eosin (phloxine) by the eosin-methylene blue method after fixation in Zenker's fluid. They stain even more intensely by the following simple method after fixation in alcohol (80 to 95 per cent).

Phloxine Stain for Alcoholic Hyalin

1. Stain paraffin or celloidin sections rather lightly in alum hematoxylin.
2. Wash in water.
3. Stain in a 0.5 per cent solution of phloxine in 20 per cent alcohol for 1 or more hours. Place in incubator at 37° C if necessary.
4. Wash in water.
5. Transfer to a very dilute solution of ammonia water (1 drop of a strong (28 per cent) solution to 100 cc. of water) for 1 to 2 minutes.
6. Wash in water.
7. Decolorize in 95 per cent alcohol, followed by absolute alcohol and xylol for paraffin sections or by oleum origani cretici for celloidin. Mount in Canada balsam.

Nuclei blue, hyaline reticulum and granules intense pink to bright red.

The granules and reticulum also stain deeply by the Macallum³ and Fiske-Subbarow⁴ methods for phosphates. Both methods depend on the formation of a phosphorus-molybdenum compound that is rendered prominent through its reduction by certain reagents to the blue oxide of molybdenum.

Macallum Test for Phosphates, as Used in these Experiments

1. Alcohol fixation (80-95 per cent): celloidin embedding.
 2. Place section in following mixture in incubator at 37°C for 24-48 hours. Make two solutions. Add A to B slowly with constant agitation to prevent formation of insoluble precipitate.
- | | |
|---------------------------------------|-------|
| A. Molybdic acid (H_2MoO_4) | 1 gm. |
| (Do not use anhydride form, MoO_3) | |
| Ammonia water, S.G. 0.88 | 4 cc. |
| Water | 4 cc. |
- Stir until salt is dissolved.

- B.* Nitric acid, S.G. 1.2 (33 per cent) 15 cc.
 Water 15 cc.
3. Wash in water 1-2 minutes.
 4. Place in a 2 per cent aqueous solution of phenylhydrazine hydrochloride for 2-10 minutes.
 5. Wash in water, 95 per cent alcohol, origanum oil, balsam.

Fiske-Subbarow Test for Phosphates

1. Alcohol fixation: celloidin embedding.
2. Two solutions are necessary.
 - A.* 2.5 per cent ammonium molybdate made up in 5 N sulphuric acid.
 - B.* 0.25 per cent aqueous solution of aminonaphtholsulphonic acid.
3. Place sections in a mixture of the two solutions, combined in the proportions of 10 cc. of *A* to 4 cc. of *B*, in the incubator at 37° C for 24-48 hours.
4. Wash in water, 95 per cent alcohol, origanum oil, balsam.

Certain clinical aspects of alcoholic cirrhosis are of importance. It is very rare before the age of 25 years and is most common in the fourth, fifth and sixth decades. This late occurrence of the process, although indulgence in liquor often begins much earlier, argues that the condition is of slow development, requiring ordinarily some five to fifteen years or more for its production. On the other hand, it sometimes occurs in children and it has been noted as occasionally affecting more than one child in a family. I have studied sections from the livers of a girl 12 years of age and of her brother aged 17 years, both of whom died at the Massachusetts General Hospital from typical chronic alcoholic cirrhosis. No history of indulgence in alcohol was obtained and it is unlikely to have occurred at that early age.

One gains the impression from the study of the disease that it is much more common among the poorer people who drink the cheaper, less carefully prepared and less aged brands of liquor.

Although investigators are generally agreed that alcoholic cirrhosis is not due to ethyl alcohol (all experimental work is against it) it is well recognized that there is a very close relation between

alcoholic beverages and the disease. This fact suggests strongly that the injurious agent is a contaminating substance contained at times in liquors.

PHOSPHORUS CIRRHOSIS

Much attention has been paid to the marked fatty changes and necrosis produced in the liver by acute poisoning with phosphorus, and several observers have stated that the cytoplasm of the liver cells is more affected than the nuclei. Chronic poisoning on the other hand has attracted practically no notice and Wegner⁵ (1872) seems to be the only experimenter on record who has produced cirrhosis in this way.

In my experimental work the greatest difficulty has been to determine the dosage and time required to produce the effects desired. So far 85 rabbits and guinea pigs have been used. The results obtained can be summarized fairly briefly.

Cirrhosis of the liver can easily be produced by phosphorus in three months or more (Figs 11 and 12) and is often complicated by ascites and jaundice. The daily dose required is two-thirds to one milligram of phosphorus to each kilogram of body weight. A 0.2 per cent solution of yellow phosphorus dissolved in oil of sweet almonds is convenient to use because 1 cc. equals 2 mg. of phosphorus. By means of a small syringe the exact amount required for each animal is readily measured and then injected slowly into the mouth of the animal held in the vertical position. The solution causes no discomfort or irritation. For the more chronic type of lesion a 0.1 per cent solution will be found preferable.

Phosphorus produces apparently two effects on the liver. It injures two different types of cells, the fibroblasts of the stroma, especially in the region of the portal vessels (Fig. 11), and the liver cells throughout the lobule. Destruction of fibroblasts is followed by regeneration, as shown by the occurrence of occasional mitoses, and by fibrosis, which is periportal in the beginning but soon extends irregularly into the lobule (Fig. 13). The fibrosis of the liver is easily produced, leads to marked increase in consistence and gives the liver a finely granular surface, usually most noticeable on its under surface.

If the dose of phosphorus is large (1 mg. to 1 Kg. of body weight), the liver cells are destroyed in great numbers and regeneration is

very active. Mitotic figures are numerous. If the daily dose is reduced to about 0.33 mg. to 1 Kg. of body weight, degenerative changes slowly take place. Eosinophilic granules appear in the cytoplasm, enlarge and fuse together to form a hyaline reticulum that stains acidophilically. The shortest time required to produce the degenerative changes characteristic of alcoholic cirrhosis was four and a third months (Figs. 8, 9 and 10). Even in this liver mitotic figures were present (Fig. 10). A smaller dose would seem to be preferable (0.25 mg. to 1 Kg. of body weight), but six months to a year or more might be required to produce well marked lesions.

Satisfactory degenerative changes are much more difficult to produce than the fibrosis because a smaller dose and much longer time are required.

Attempts to produce similar degenerative changes in liver cells by the use of certain poisonous compounds of phosphorus, such as ethyl and diethyl phosphine and calcium phosphide, gave only negative results. For their preparation and the opportunity to test them I am greatly indebted to the kindly interest of Dr. Harry M. Weber, chemist.

The lesion characteristic of alcoholic cirrhosis would seem to be due to the element yellow phosphorus itself and not to any of its compounds.

DISCUSSION

As already stated, the diagnosis of alcoholic cirrhosis is based on a characteristic degenerative change occurring in the cytoplasm of the liver cells. It consists of a hyaline reticulum formed from granules that enlarge and coalesce. The granules and the reticulum are strongly acidophilic. They also give the reaction for phosphates by the Macallum and Fiske-Subbarow microchemical staining methods.

It is possible by the daily administration of very minute doses of yellow phosphorus dissolved in oil of sweet almonds to produce a definite cirrhosis in rabbits and guinea pigs in three months or more. If the dosage is made smaller and continued over a longer time, so that the liver cells degenerate very slowly instead of undergoing necrosis, a series of retrograde changes similar to those found in alcoholic cirrhosis and reacting microchemically in the same way

slowly takes place. Acidophilic granules appear in the cytoplasm, enlarge and fuse to form a hyaline reticulum.

These experimental results afford morphological and microchemical evidence that alcoholic cirrhosis may be due to phosphorus. The natural question is — how can the patient take phosphorus into his system? One possible way is obvious. Phosphorus is administered therapeutically chiefly under three conditions, (1) in rickets, (2) to promote callus formation, and (3) in certain nervous disorders. Its use in rickets may possibly explain the rare occurrence of alcoholic cirrhosis in children, but certainly throws no light on the frequent occurrence of the disease in adults.

All available evidence points strongly to the conclusion that this most common type of cirrhosis is connected in some way with excessive indulgence in strong liquors. Is it possible that they sometimes contain phosphorus?

Naturally the greatest objection to the suggestion of phosphorus as the cause of alcoholic cirrhosis lies in the difficulty of explaining how it can get into alcoholic beverages — its probable source. Evidently it is not deliberately put there. If it is ever present, it must be as an accidental contaminant. Up to the present time only one possible source has suggested itself and that is iron.

Phosphorus is present in certain iron ores, sometimes in considerable amount, and thus gets into pig and cast iron and also into iron and steel made from them. It is said to exist there as iron phosphide and also as an alloy of iron. Iron utensils and cans made from tin plate might be conceived therefore as yielding phosphorus if eroded by acids, which are commonly present in liquors. If set free, the phosphorus would probably be preserved, at least for some time, in water and even better in alcohol just as it is in oils. This idea was adopted as a working hypothesis and various substances including a limited number of liquors tested.

Tests for Phosphorus: Phosphorus is characterized chiefly by two properties, phosphorescence and its reaction with silver salts. Unfortunately neither is diagnostic. Phosphorescence was discarded early in this experimental work because it does not afford a sufficiently delicate test and is prevented by the presence of alcohol.

The silver test employed in toxicology is performed by suspending a strip of paper moistened with nitrate of silver in the neck of a flask containing the suspected fluid. The apparatus is warmed gently at a

temperature of 30 to 40°C (according to Autenrieth 40–50°C) for several minutes. The flask is closed by a perforated cork to permit the slow escape of vapor, which is carried over the surface of the paper. Phosphorus turns the paper light to dark brown (silver phosphide). Hydrogen sulphide, however, produces much the same effect (silver sulphide). Therefore a second strip of paper moistened with acetate of lead is also suspended in the flask. If only the silver paper is browned, phosphorus is present; if both papers are browned, hydrogen sulphide is present and phosphorus may be. No method for differentiating the effects of these two substances on the silvered paper from each other is given. An attempt to find one resulted in the following.

Photographic printing paper was found to be more convenient and sensitive than the paper moistened with silver nitrate. It is turned light to dark brown in a few minutes to 24 hours by phosphorus, depending on its strength, and light to dark brown by hydrogen sulphide. Placed in a solution of permanganate of potassium acidified with sulphuric acid (equal parts of a 0.25 per cent solution of the first and of a 2 per cent solution of the acid), the paper is decolorized in 1 to 5 minutes, depending on the intensity of the color. The phosphorus compound is the more readily changed. After washing in water the paper, now colored brownish by the potassium permanganate, is placed in the Fiske reagent which promptly decolorizes it and, if phosphorus is present, gradually turns it blue in the course of 5 to 10 minutes. Wash in water, place in the ordinary hypo (sodium thiosulphate) solution for 2 to 5 minutes to remove any soluble silver salts present, wash thoroughly in water and dry. If hydrogen sulphide was present, the paper may be colored a very light brown. After the first step, which is best performed in the dark, the test may be carried out in ordinary light.

The method, which was arrived at only after much experimentation, was tried out first with water saturated with phosphorus (1:33,000) using a 24 hour exposure of the paper to the vapor at a temperature of 25°C. Diluted 1:10 (1:330,000) a positive reaction could still be obtained and alcohol did not interfere with the test.

Tested in the same way, 10 per cent hydrochloric acid gave a negative result, as did also chloride of iron, iron sulphide and iron phosphide, all of which were dissolved in 10 per cent hydrochloric acid. On the other hand, a strongly positive reaction was obtained

with cast iron chips, C. P. iron (alcoholized, Eimer and Amend), and tin plate (cut into small bits so as to expose as much iron surface as possible), all dissolved in 10 per cent hydrochloric acid.

These reactions would seem to indicate that phosphorus is commonly alloyed with iron and is set free as the element when iron is dissolved in an acid, or at least in dilute hydrochloric acid. It evidently is not derived from the iron phosphide.

Up to the present time the test has been applied to 25 samples of hard liquor with entirely negative results.

Certain technical points in connection with the demonstration of phosphorus may be of use. I have employed Gevaert's Nova Brom photographic printing paper almost exclusively in these experiments.

If the paper is developed with a metol-hydroquinone solution after exposure to phosphorus very minute quantities may be demonstrated. The same is true of hydrogen sulphide. The method, however, is much more delicate than that employing silver nitrate paper or than the Fiske reaction. Possibly it may be developed into a diagnostic test later.

Developed by the metol-hydroquinone solution, the paper acted on by phosphorus turns gray to intense black, while that exposed to hydrogen sulphide retains a brownish tinge.

The acid permanganate and Fiske solutions should be combined immediately before use and not employed for a second lot of paper.

SUMMARY AND CONCLUSIONS

Very chronic poisoning with minute daily doses of yellow phosphorus produces, in five months or more, cirrhosis of the liver in rabbits and guinea pigs.

The liver cells undergo degenerative changes similar to those found in alcoholic cirrhosis. They contain granules and hyaline networks (reticula) that are acidophilic and give the reaction for phosphates by the Macallum and Fiske-Subbarow tests.

By a special method it is possible to demonstrate free phosphorus when cast iron and tin plate are dissolved in dilute hydrochloric acid.

Phosphorus may get into alcohol and liquors through acid erosion of iron and tin plate vessels and containers and thus contaminate them and be the cause of alcoholic cirrhosis.

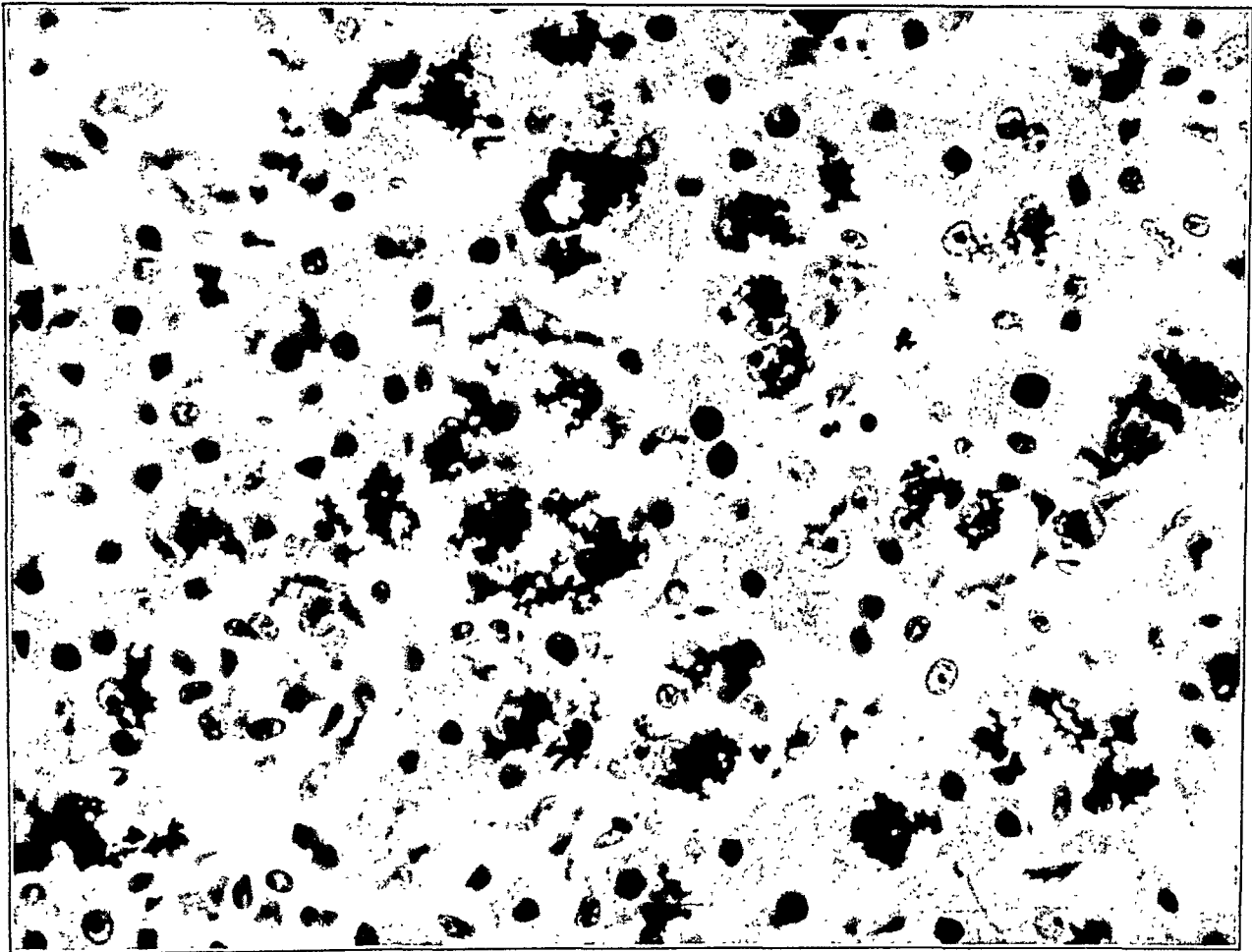
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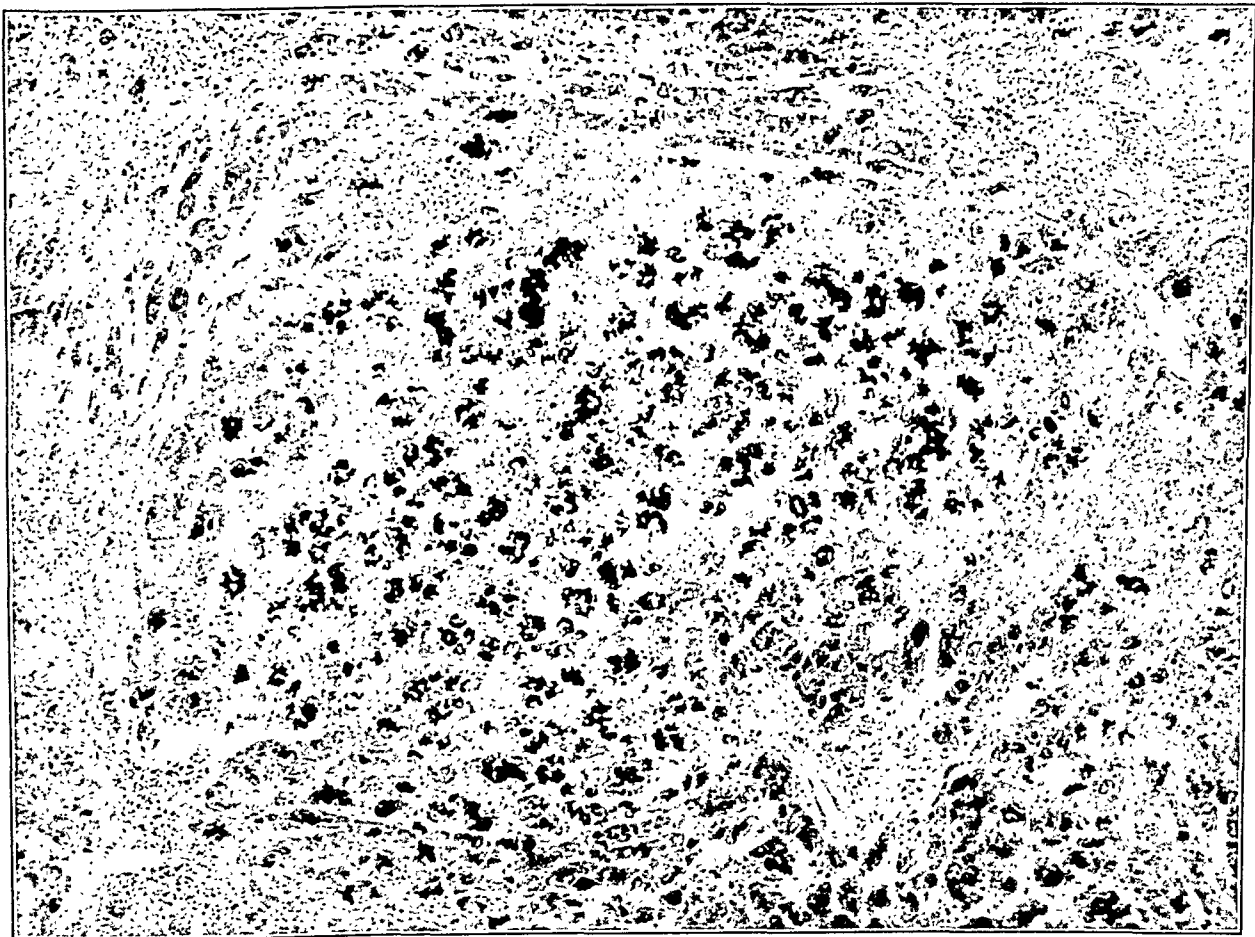
DESCRIPTION OF PLATES

PLATE 88

- FIG. 1. A-97-172. Alcoholic cirrhosis; first case observed and studied. Many of the cells contain coarse masses of acidophilic material (hyaline network or reticulum) in their cytoplasm. $\times 500$.
- FIG. 2. U-30-33. Alcoholic cirrhosis — a chronic case. Nearly every liver cell in an island of regeneration is filled with fairly coarse hyaline material. $\times 100$.



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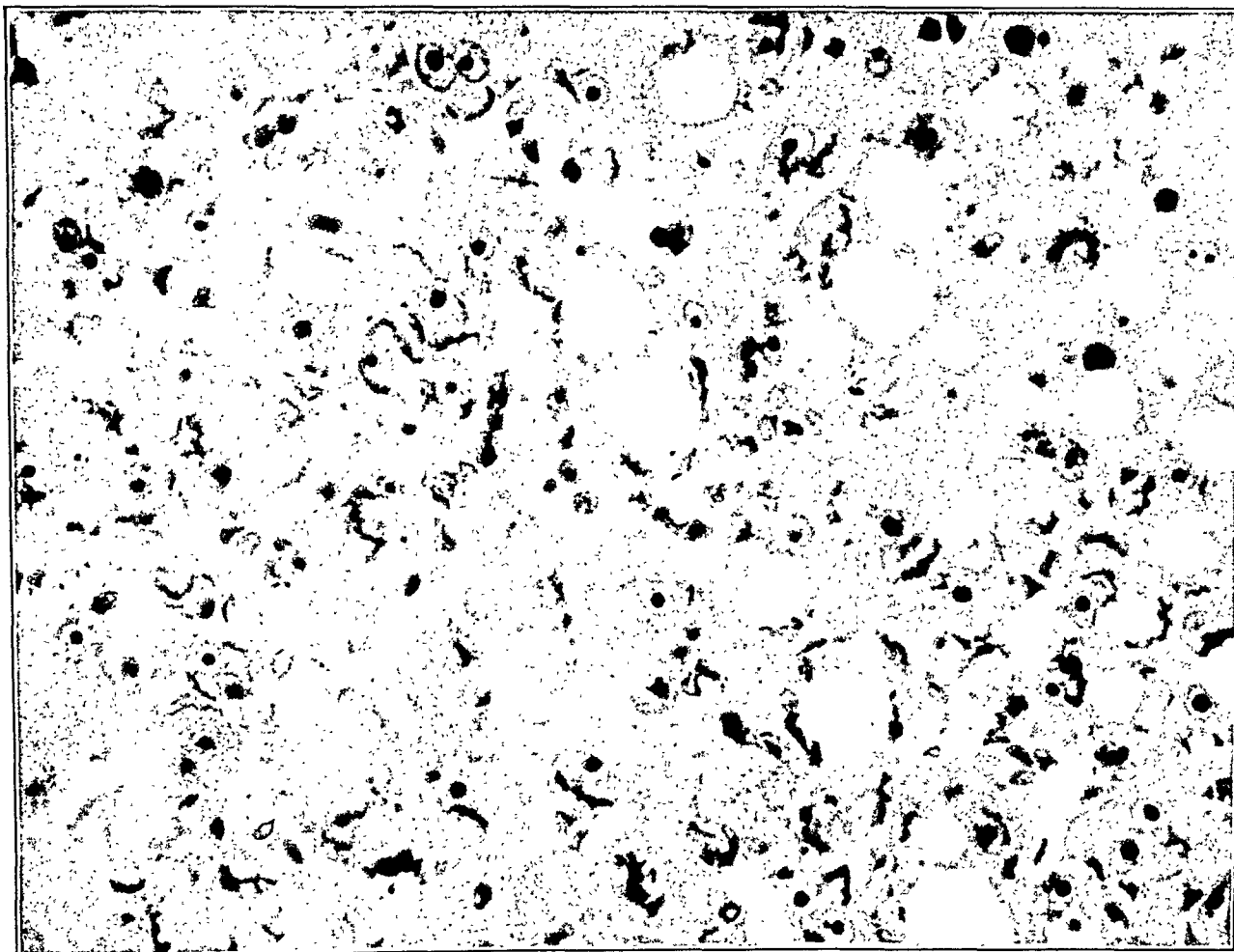


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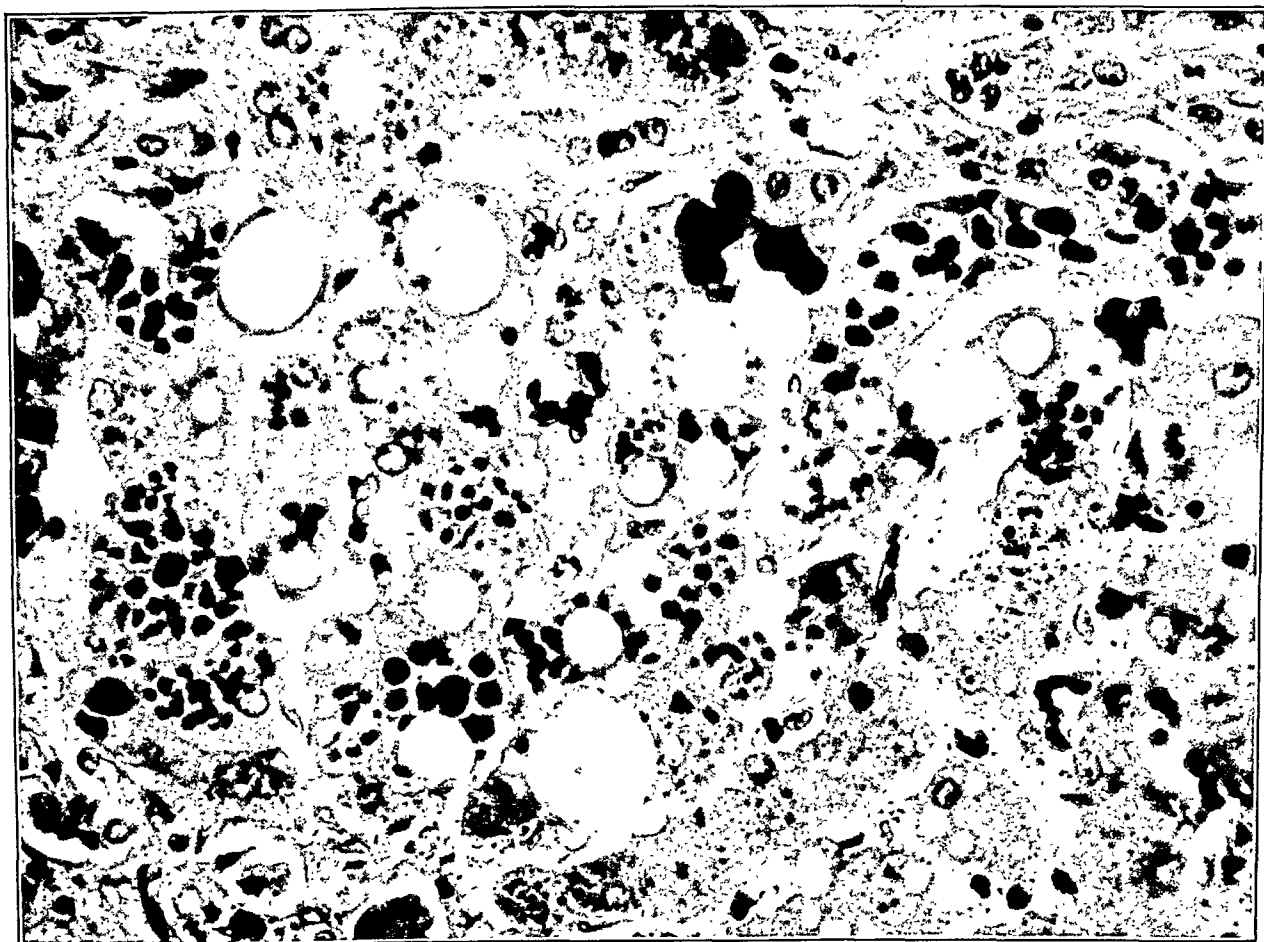
PLATE 89

FIG. 3. A-01-21. Alcoholic hyalin present in nearly every liver cell in the form of granules and delicate reticula. No cirrhosis evident. The lesion is probably of very slow formation. $\times 500$.

FIG. 4. U-27-10. Bile stasis. The inspissated bile in the cytoplasm of the liver cells is in the form of variously sized droplets and has been decolorized and become acidophilic so that it stains deeply with eosin. $\times 500$.



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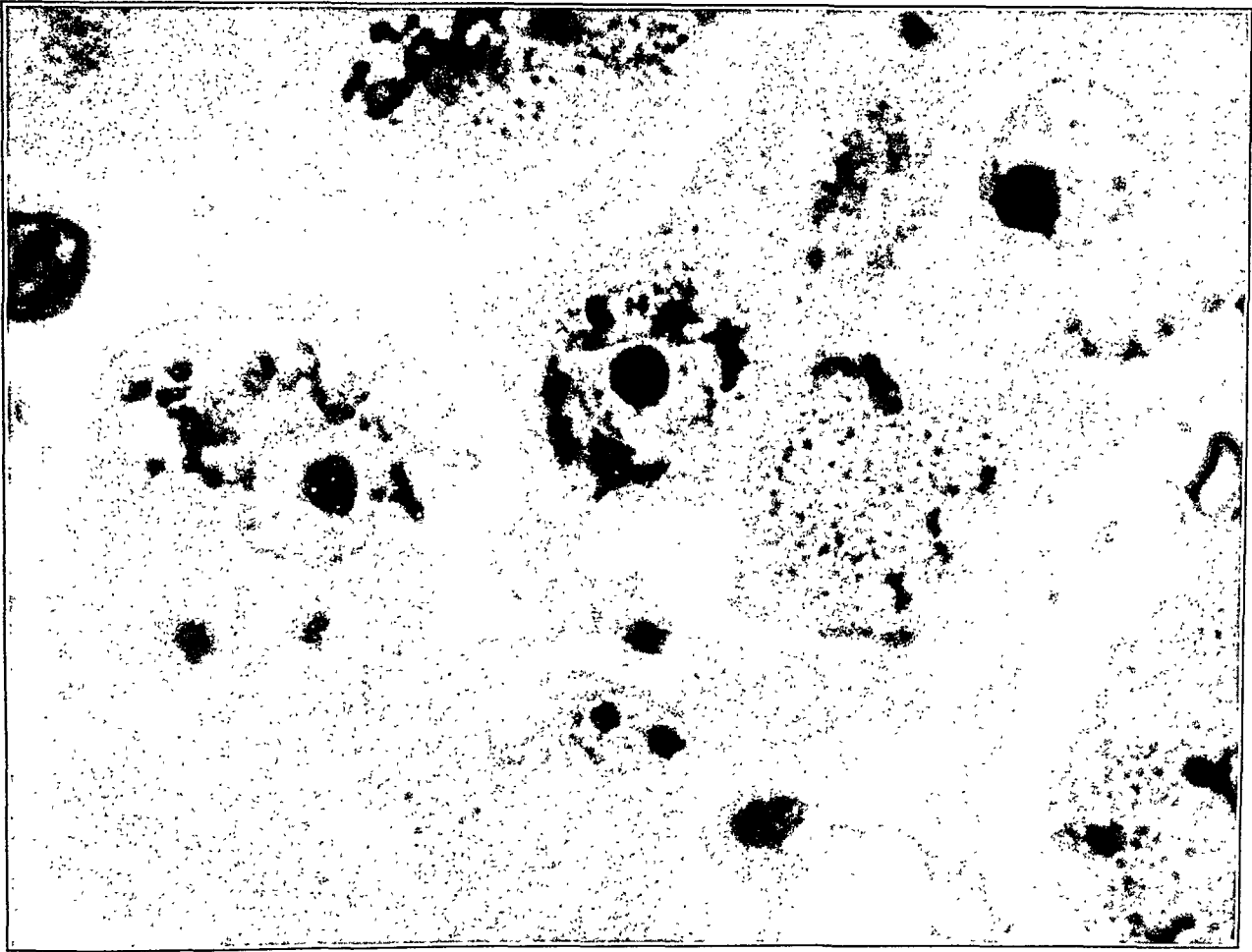


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PLATE 90

FIG. 5. A-01-21. Alcoholic hyalin in the process of development from eosinophilic granules which enlarge and coalesce to form a network in the cytoplasm, sometimes surrounding the nuclei. $\times 2000$.

FIG. 6. A-01-21. Another example of hyalin forming from eosinophilic granules in the cytoplasm of liver cells. $\times 2000$.



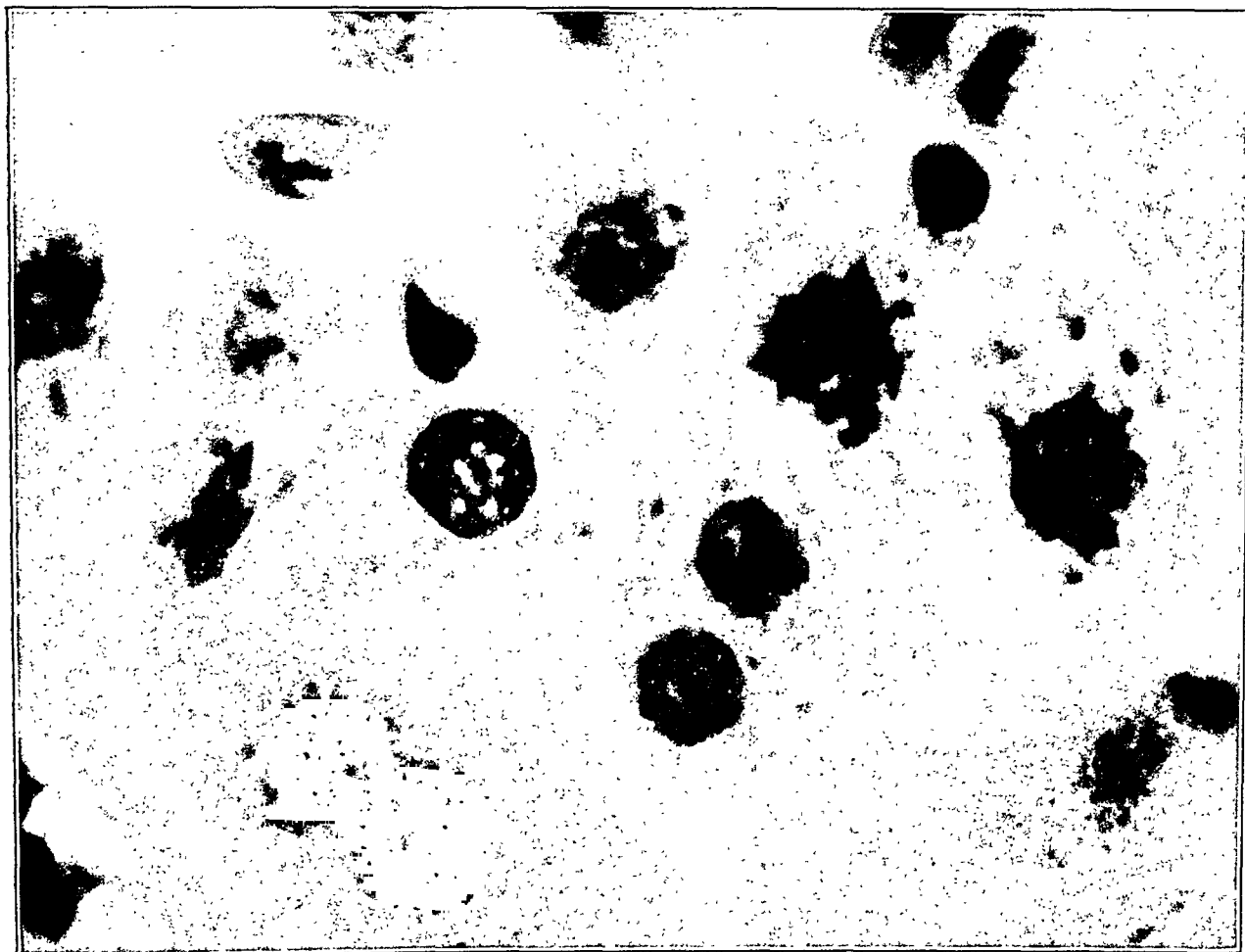
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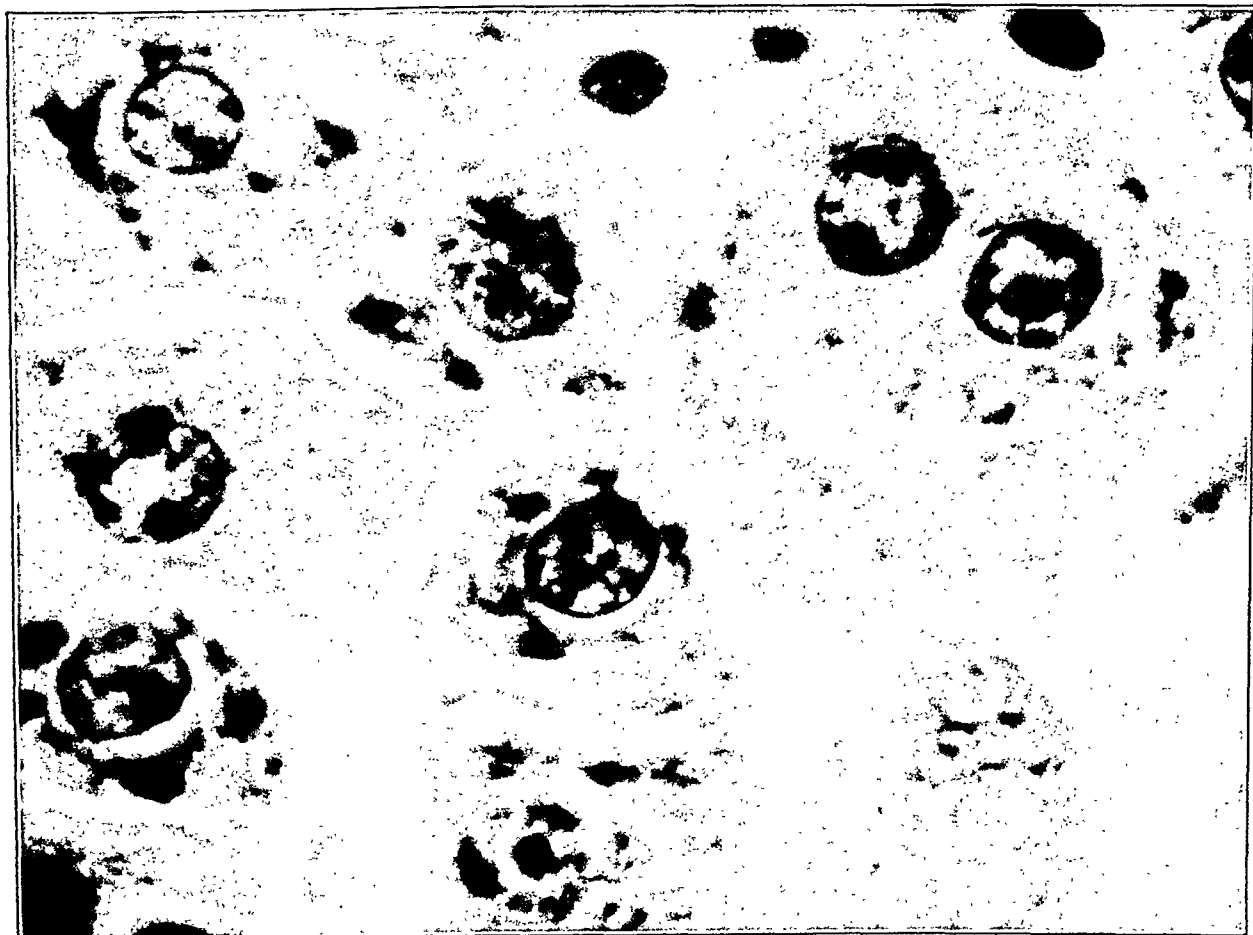
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PLATE 91

- FIG. 7. Z801. Phosphorus cirrhosis, $4\frac{1}{3}$ months duration. Average daily dose of phosphorus 0.25 mg. to 1 Kg. of body weight. Early stage showing eosinophilic granules beginning to appear, enlarge and fuse to form hyaline material. $\times 2000$.
- FIG. 8. Z780. Phosphorus cirrhosis, $4\frac{1}{3}$ months duration. Dose of phosphorus given daily 0.33 mg. to 1 Kg. of body weight. Every cell contains hyaline material in the process of forming a network more or less surrounding the nuclei. $\times 2000$.



7

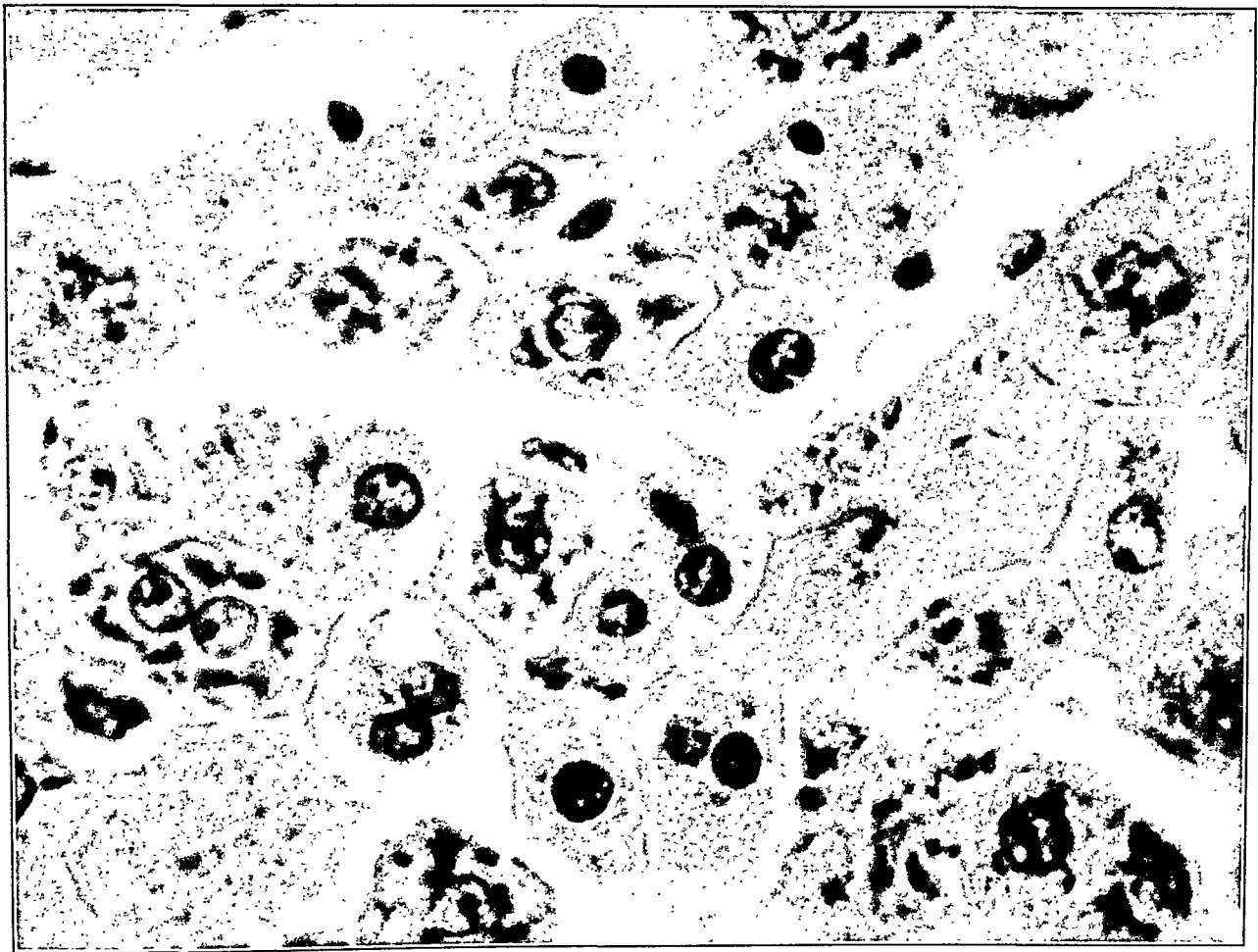


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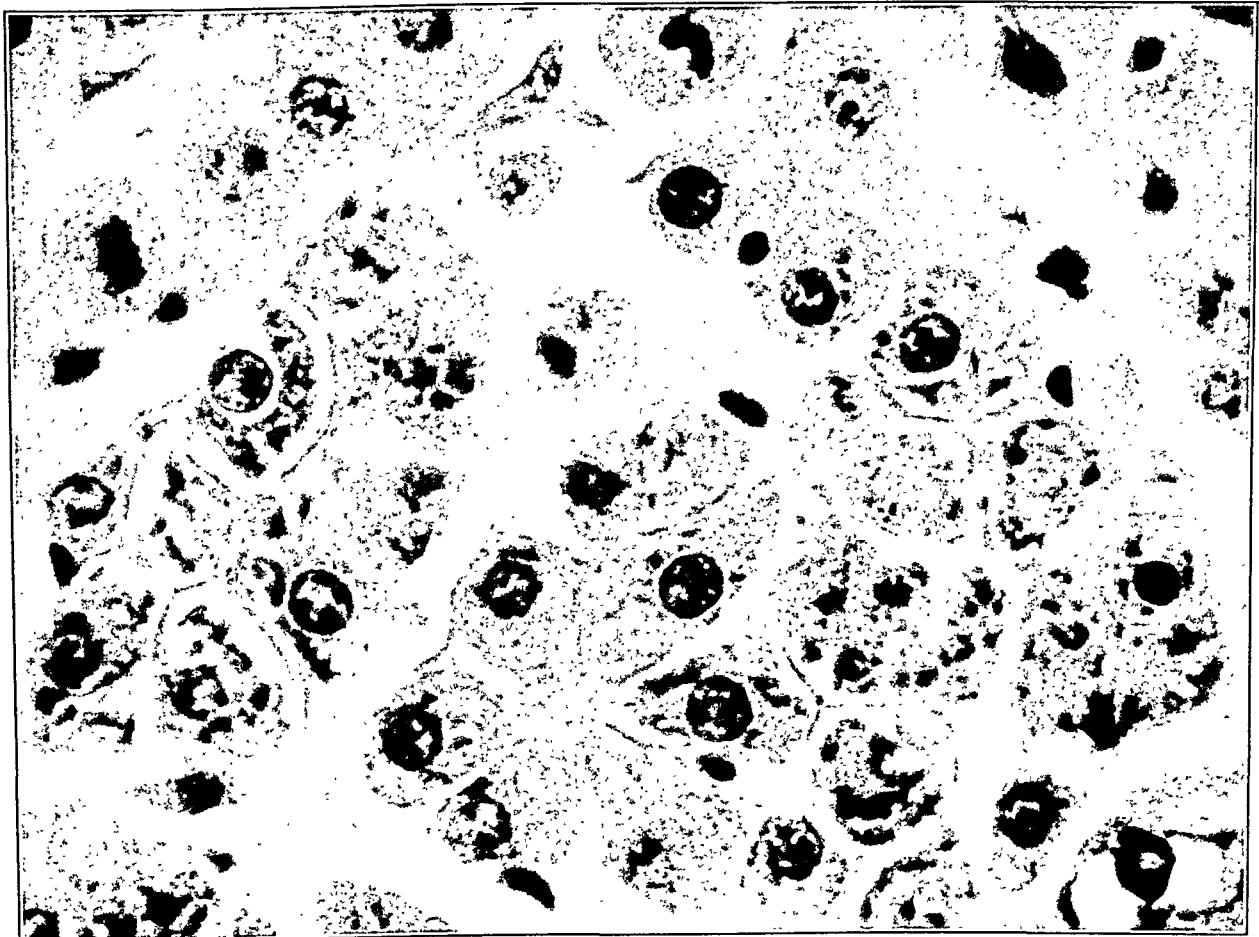
PLATE 92

FIG. 9. Z780. Phosphorus cirrhosis. Nearly every cell contains granules and hyaline reticula that stain intensely with phloxine. $\times 1000$.

FIG. 10. Z780. Another field from the same liver as in Fig. 9. Two mitoses are present, one in the upper center and the second in the upper left corner. $\times 1000$.



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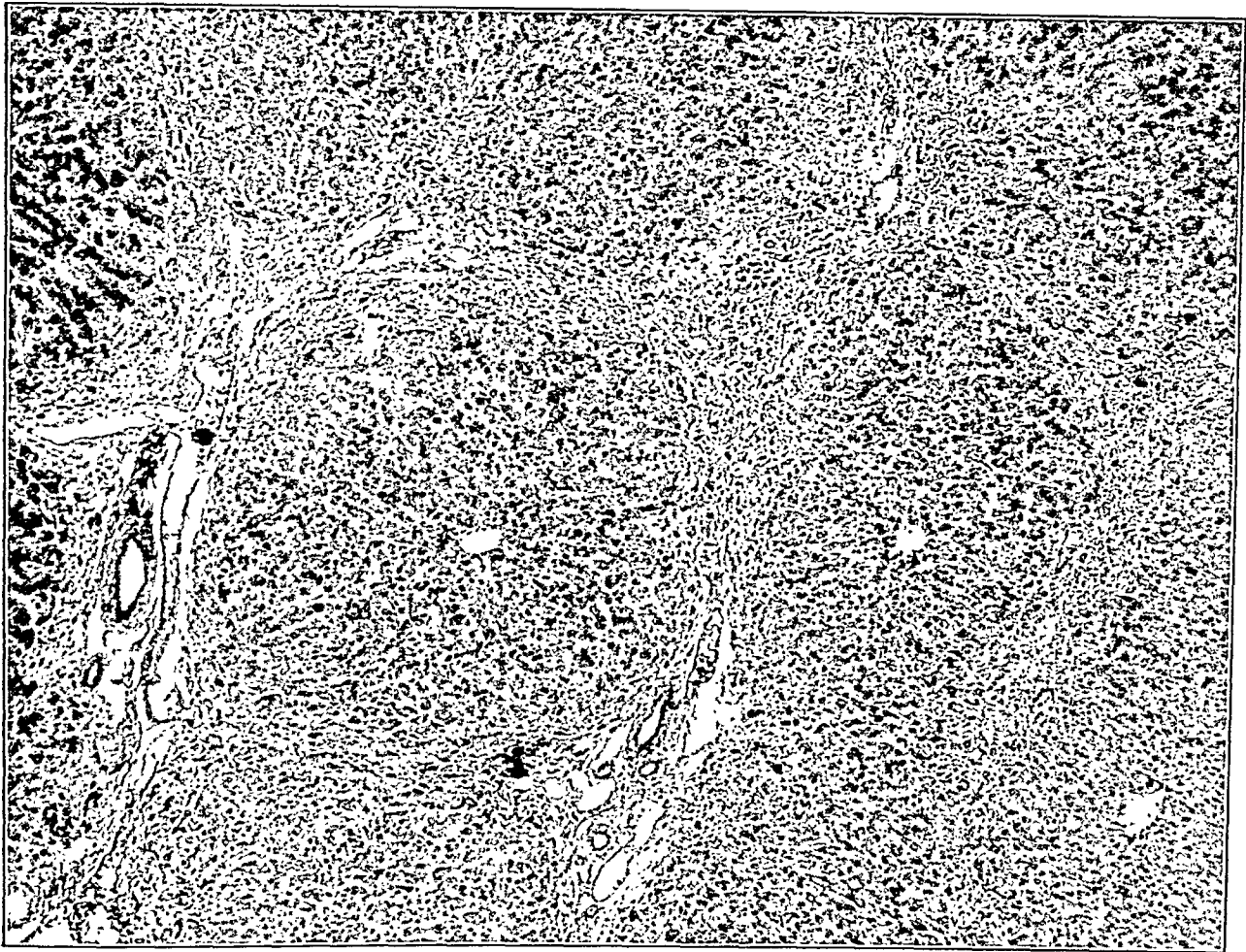


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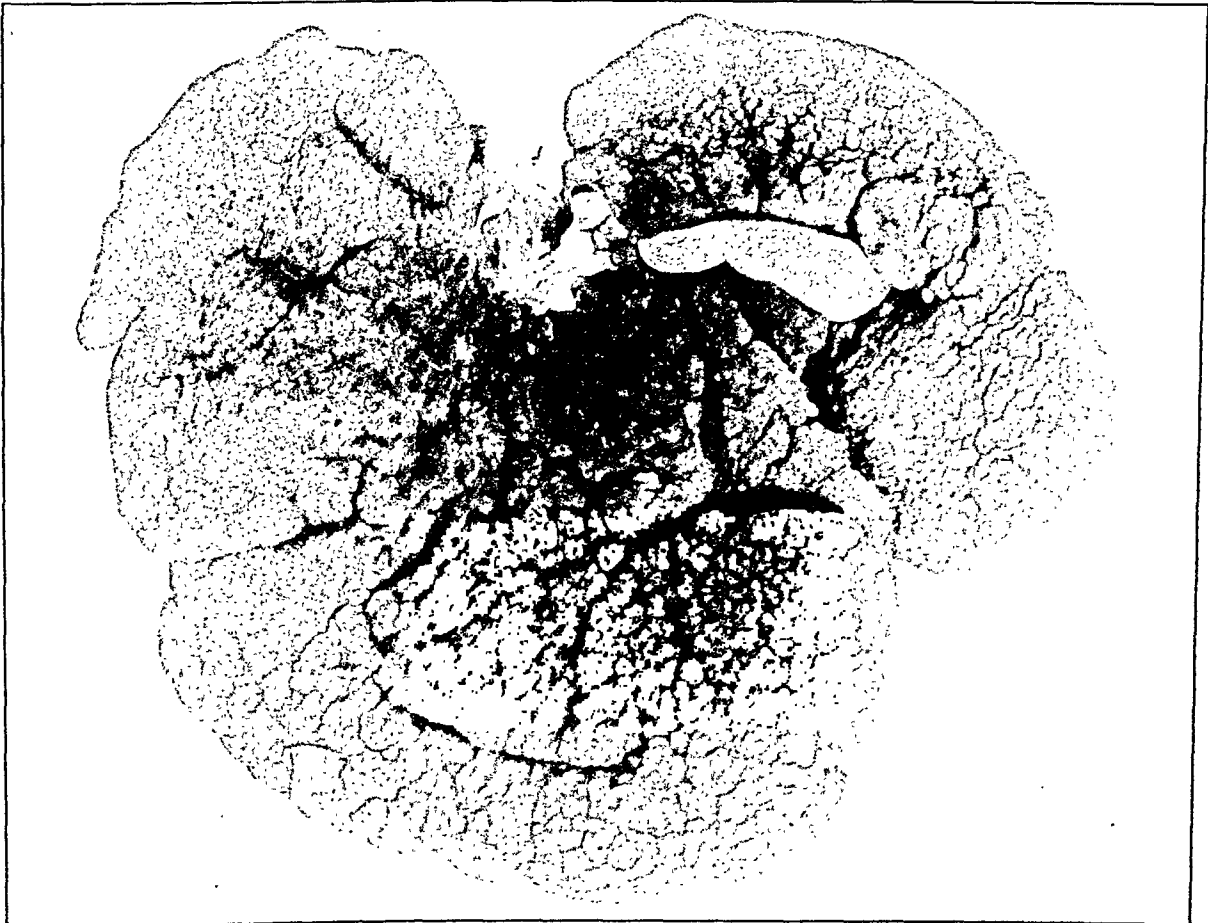
PLATE 93

FIG. 11. Z737. Phosphorus cirrhosis produced in 8 weeks. Daily dose 1 mg. to 1 Kg. of body weight. Cirrhosis distinctly periportal in origin and distribution. $\times 50$.

FIG. 12. Z738. Phosphorus cirrhosis produced in 11 weeks. Similar in character to Z737 (Fig. 11).



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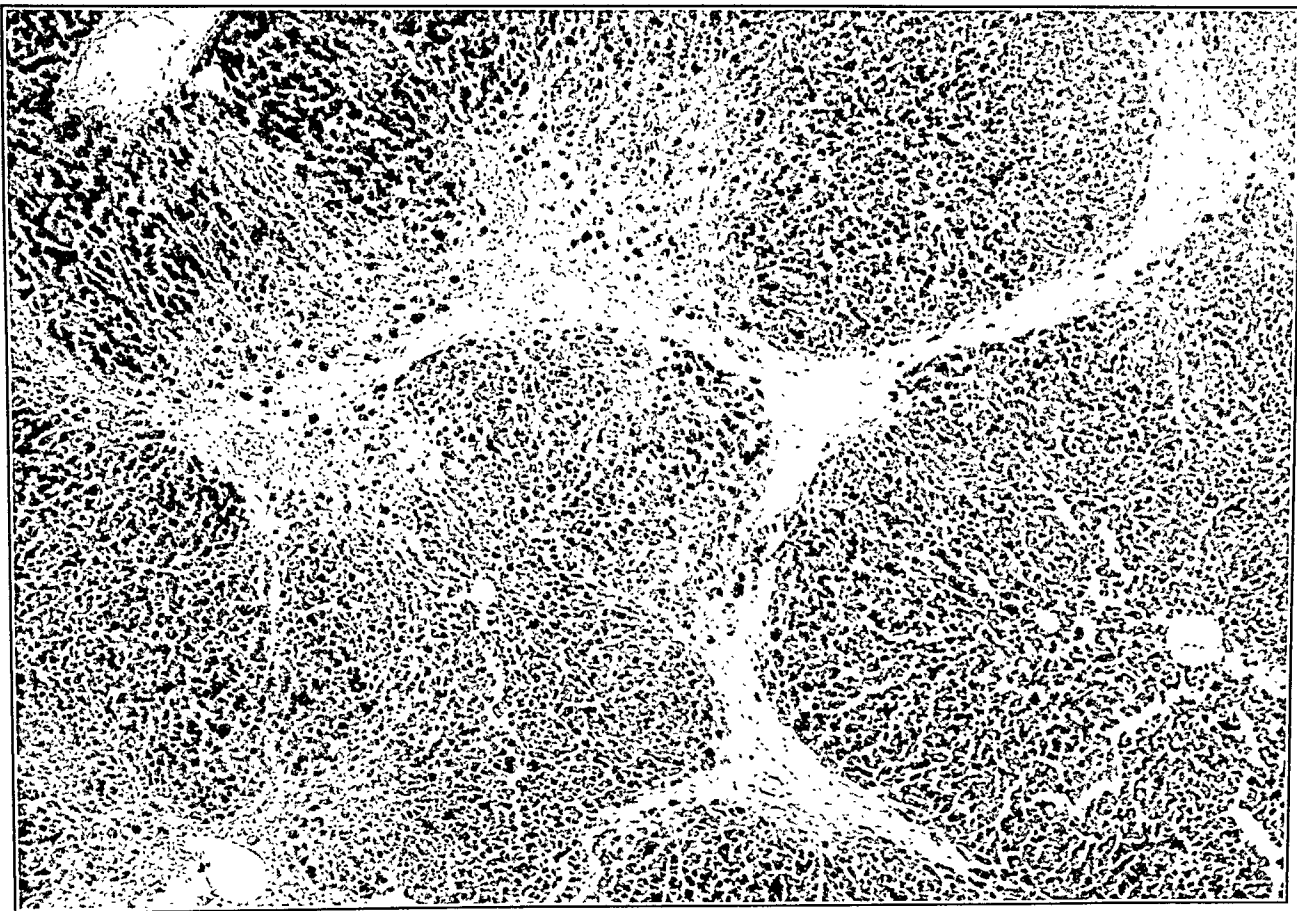


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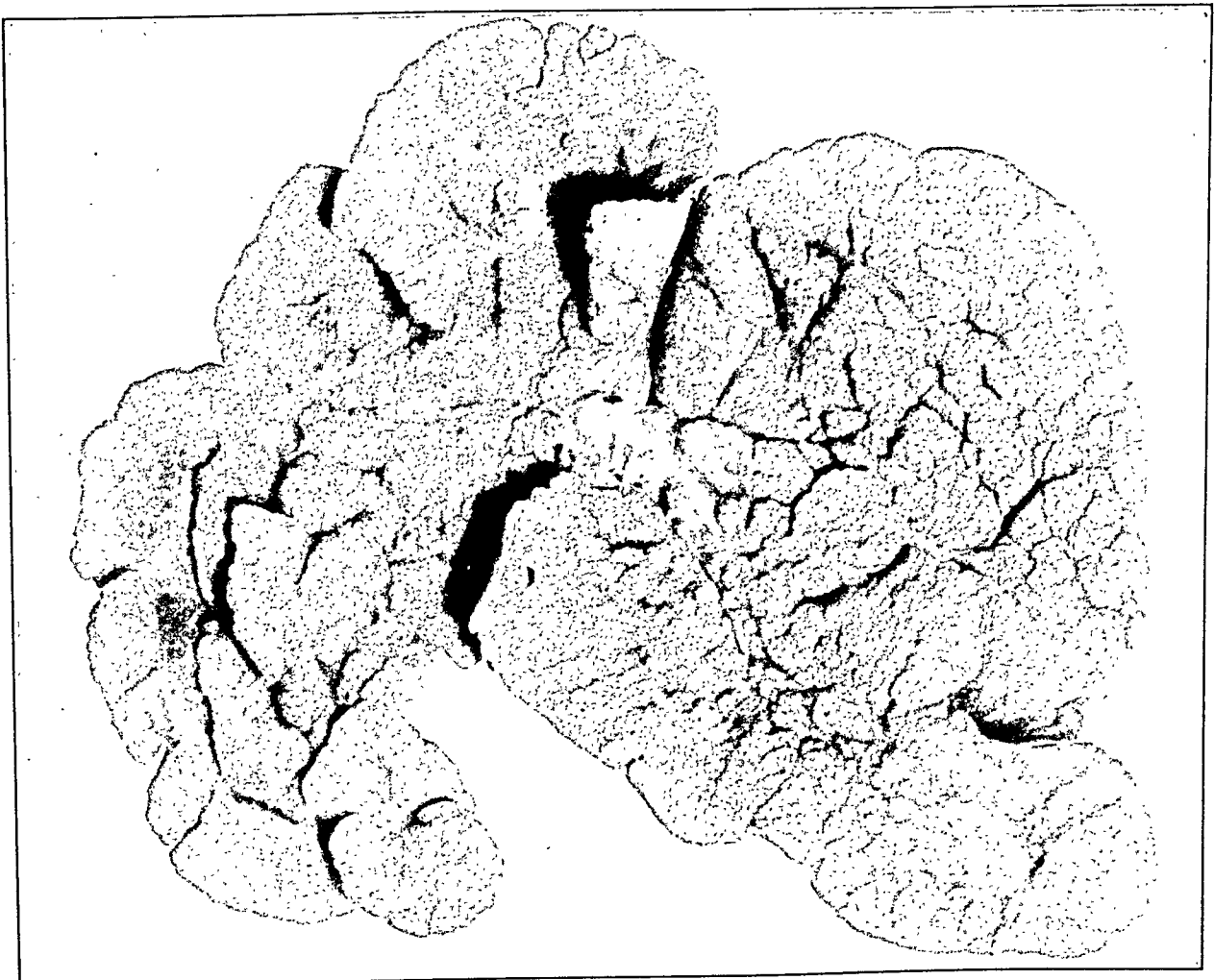
PLATE 94

FIG. 13. Z780. Phosphorus cirrhosis. Low power view to show the degree of sclerosis present. It is periportal in origin but is extending irregularly into the lobules. Details from this liver are pictured in Figs. 8, 9 and 10. $\times 50$.

FIG. 14. Z780. Phosphorus cirrhosis. Gross appearance of under surface of liver.



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THE PERMEABILITY OF THE KIDNEY TO BACTERIA *

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In spite of considerable experimental investigation there is still doubt as to whether or not the normal uninjured kidney can discharge bacteria coursing in the circulation. Although it is true that the majority of authors contend that such an "excretion" occurs only after definite lesions have been produced in the kidney, one constantly encounters even in the recent literature such statements as "the ability of the normal kidney to excrete any organisms that may, from time to time, reach the blood-stream." The present study is a reexamination of the problem with particular emphasis on the exact localization of the bacteria in the kidney after their injection into the circulation.

The first published statement on the subject was that of Cohnheim,¹ who said that "not only the dissolved constituents of the blood, *but some undissolved ones* (including bacteria) *are capable of excretion by the kidneys*, and then make their appearance in the urine secreted by healthy kidneys when and because they are contained in the blood." This opinion was soon challenged by Wyssokowitsch (1886),² who found that various spores and bacteria which did not affect the kidneys were absent from the urine for periods up to 24 hours after injection into the blood stream of dogs and rabbits. Even *Staphylococcus aureus* and *B. anthracis* did not appear until several hours after injection, when the kidneys were already the seat of recognizable lesions. He used different methods of obtaining the urine. During life the animals were catheterized; after death, in males, the urine was expressed from the bladder through the urethra, while in females the abdomen was opened, a hole burned through the bladder wall and a pipette inserted.

In 1887 Schweizer,³ using a "green-producing bacillus" for injection and a ureteral fistula for obtaining the urine, found the organisms in the urine frequently within a few minutes after their intro-

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duction into the blood stream. He suggested that bacteria settling in the kidney might damage a few cells in such a way as to permit their escape into the urine, although such lesions were not demonstrable in stained sections. Sherrington (1893),⁴ in a careful study of the presence of bacteria in several of the body secretions, showed that they occurred only after an interval sufficient to result in damage to the secreting membranes.

The article by Biedl and Kraus,⁵ which appeared in 1896, has been an important factor in fortifying the opinion that the kidney can readily excrete bacteria. These workers injected *Staphylococcus aureus*, *B. coli* and *B. anthracis* into dogs and rabbits and, on culturing the urine, often found the organisms 5 to 15 minutes after the administration. Their usual method of recovering the urine was by cannulae inserted into the ureters. Their results were far from consistent and they were careful to point out that there was nothing regular in the appearance of the bacteria in the urine, as one might expect in a physiological process. Their experiments were confirmed by von Klecki⁶ and Opitz,⁷ although the latter raised several important theoretical considerations against the view that the normal kidney could excrete bacteria.

Dyke (1923)⁸ studied the changes in the kidney taking place at various intervals after the intravenous injection of *Staphylococcus aureus* and, although he could demonstrate a reaction of the tissues as early as 1 hour after the injection, the cocci did not appear in the urine till the 5th hour; by this time there were very definite inflammatory changes in the kidney. In a thorough and convincing series of experiments Helmholtz and his associates⁹⁻¹⁵ showed that various organisms, virulent and avirulent, did not pass into the urine within the time usually considered adequate for excretion, but that inflammatory changes and mechanical interference with the urinary system could break down the kidney filter for bacteria.

The above is only a representative selection of writers who have performed experimental investigations of this problem, but it serves to indicate the contradictory results and opinions. Comparatively few attempts have been made to reconcile the different results. Jahn¹⁶ found that the urine of a number of species of mammals at times had a bactericidal effect on some of the common pathogenic organisms. He suggested, therefore, that those urines giving negative results within a few minutes after the intravenous

injection of bacteria may have had a destructive effect on the small number of organisms that had passed through. Lepper¹⁷ thought that the manipulation necessary to secure the ureteral catheters which were used by Biedl and Kraus and by Opitz may have led to a temporary increase in pressure in the pelvis of the kidney. This would cause compression of the renal veins, congestion of the kidney and consequently slight hematuria. The most significant work in this connection was that of Helmholtz and Field.¹¹ They compared the results of their first series of experiments, in which the urine was obtained through a seared portion of the bladder wall, with a second group in which the urine was withdrawn through a catheter and was thus liable to contamination by blood, due to the trauma of the catheterization. In the latter the urine contained organisms in the first 2 or 3 hours after injection in nearly 50 per cent of cases. Also, the irregularity in time of appearance and number of bacteria in the second series is strongly suggestive of the findings of Biedl and Kraus and of von Klecki.

A careful study of the literature inevitably leads to the conclusion that the crux of the matter, in so far as the experimental investigations are concerned, is the method of obtaining the urine. It is readily apparent that any procedure in which there is any possibility of contaminating the urine with blood is liable to lead to false conclusions. This is particularly true in the first few minutes after injection, when the blood is teeming with organisms. And, of course, this is just the period when the recovery of the bacteria in the urine makes the "excretion" seem so similar to that of a dye like methylene blue, which appears in the urine within 2 or 3 minutes after introduction into the circulation. Some workers have attempted to rule out contamination by blood by examining the urine chemically, but this is open to the objection that the usual tests are not sufficiently delicate to detect a minute amount of blood which might nevertheless contain a considerable number of bacteria.

Just how much the various operative procedures, anesthesia, presence of toxic material in the broth of cultures, number of organisms injected and other factors affect the kidneys is difficult to judge, but it seems safe to assume that the less interference with the animal in the interval between the injection of the bacteria and the recovery of the urine the more likely that the results will be reliable. On this account, after a thorough analysis of the many variations in tech-

nique previously employed, the writer decided the method that promised most in the elimination of possible sources of error was that in which one animal was used for each experiment, and the urine obtained by cauterizing a portion of the bladder wall and withdrawing the contents with a syringe.

The examination of ordinary histological sections to determine the exact location of a few isolated microorganisms in the tissues is an exceedingly laborious task, particularly when it is realized that a cross-section of the rabbit's kidney, measuring 2 to 3 square cm., may contain only one or two bacteria. An even more important consideration is that the results of such an examination may be unreliable, as will be pointed out later. To overcome these objections, the method of first incubating the tissue, in order to permit the growth of the individual bacteria into easily discernible colonies, was adopted.

EXPERIMENTAL STUDY

Male rabbits between the ages of 6 and 12 months were used in all the experiments. Some days before the injection of the organisms the animals were catheterized and the urine examined for evidence of spontaneous kidney infection.

Three organisms were used: *Staphylococcus aureus*, *Bacillus coli* and *Bacillus prodigiosus*. The staphylococcus was obtained from a human case of osteomyelitis of a tarsal bone and the colon bacillus from a case of pyelitis in a child. To eliminate the possible toxic effects of bacterial products present in broth cultures, saline suspensions were used throughout. Each organism was grown in Hartley's broth plus dextrose for 18 to 24 hours and then suspended in physiological saline so as to form a distinctly turbid mixture. The McFarland nephelometer was used to obtain emulsions of approximately equal turbidity for experiments on different days. In order to get rid of large clumps of organisms and so avoid the possibility of embolism, the suspension was centrifuged at low speed for a few minutes before use. No attempt was made to determine the number of bacteria — it was deemed sufficient that a very large number of organisms be introduced into the blood stream.

Injection was made into the marginal vein of the ear, and after the desired interval had elapsed the animal was killed by a blow on the neck. To guard against postmortem evacuation of the bladder (a

mishap that resulted in the sacrifice of several animals before it was detected), the urethra was gently compressed between the pubis and the soft tissues overlying it by an assistant. Using aseptic technique the abdomen was opened and the bladder exposed. A relatively avascular portion of the wall was cauterized with a special instrument and the urine withdrawn through this area with a needle and syringe.

The urine thus obtained was divided into two samples. The first and larger was centrifuged at high speed for 30 minutes and the supernatant fluid drawn off and tested for albumin. A loop of the sediment was streaked on an agar plate, while the balance of the sediment was used to make an agar pour plate. To facilitate the identification of colonies, blood agar or plain agar was used in the experiments in which staphylococcus had been injected, Endo agar for *B. coli* and plain agar for *B. prodigiosus*. The sediment was also examined microscopically. The second sample of urine was used to test for possible bactericidal effect on the organism. For this purpose the urine was seeded with a loop of the suspension used for the injection and incubated at 37.5° C for a variable period, usually 24 hours, but never less than the time interval between the injection and the death of the animal. Transplants to agar plates were made immediately after the seeding and after the period of incubation. All the cultures were read after 3 days.

At the same time as the urine was obtained from the bladder the left renal vessels and ureter were tied off, the kidney removed to a sterile Petri dish and incubated. The kidneys in the staphylococcus and colon bacillus experiments were kept at 37.5° C for 18 to 20 hours, while in the experiments with *B. prodigiosus* the kidneys were incubated at 30° C for 24 hours. The right kidney was immediately placed in fixative (10 per cent formalin in the staphylococcus experiments and Zenker's solution in the others). After the period of incubation the left kidney was also fixed. Paraffin sections were cut and stained by a modification of the Gram-Weigert method.

RESULTS OF EXPERIMENTS

The findings in the bacteriological examination of the urine are collected in the accompanying tables. It will be noted that staphylococci first appeared in the urine in the 12 hour interval. There was

a striking relation between the number of organisms in the urine and the time interval after injection. In Rabbit 4 there were about 50 colonies in both plates. In Rabbit 273 the number was much increased but the individual colonies were easily seen, while in Rabbits 461 and 462 the colonies were so numerous and so small that in the pour plates they were not visible to the naked eye.

TABLE I

Bacteriological Examination of Urine at Varying Intervals after the Intravenous Injection of Bacteria in Rabbits

I. Staphylococcus Aureus

Rabbit No.	Amount of suspension injected	Interval between injection and death of animal	Organisms in urine	Survival of organisms in inoculated urine
454.....	1 cc.	10 min.	None	+
453.....	1	35 "	"	+
452.....	1	1 hour	"	+
451.....	1	2 hours	"	+
463.....	1	3 "	"	+
33.....	1	5 "	"	+
32.....	1	8 "	"	+
4.....	1	12 "	Few	+
273.....	1	18 "	Many	+
461.....	1	24 "	Innumerable	+
462.....	1	48 "	"	+

Other workers who do not support the theory of the excretion of organisms by the uninjured kidney have found staphylococci in the urine in as short a time as 5 hours after injection. Probably this difference in the time of the first appearance of the cocci is related to the degree of virulence of the strain and so to the rapidity with which lesions are developed in the kidney. A rabbit injected with 1 cc. of the suspension survived for 13 days, indicating that this strain was not very pathogenic as compared with others that kill an animal in a few hours.

In the series of animals injected with the colon bacillus the urine was negative even up to 48 hours after the injection. (The one organism in the urine of Rabbit 841 (Table II) was probably a contamination; it is difficult to conceive of a lesion in the kidney permitting a single bacterium to escape into the urine). Some other investigators have found the bacilli in the urine as early as the 7 hour

interval, so that here, too, the organism used was not very virulent. But the kidneys were damaged to some extent, even though not sufficiently to cause bacilluria: this is shown by the fact that from 12 hours onward the urine contained such abnormal constituents as granular casts and a fair number of epithelial cells. That the colon bacillus varies considerably in its ability to affect the kidney is a point noted by several writers (Lepper,¹⁷ Helmholz and Beeler¹⁸).

TABLE II

Bacteriological Examination of Urine at Varying Intervals after the Intravenous Injection of Bacteria in Rabbits

II. Bacillus Coli

Rabbit No.	Amount of suspension injected	Interval between injection and death of animal	Organisms in urine	Survival of organisms in inoculated urine
	cc.			
9.....	2	10 min.	None	+
28.....	2	30 "	"	+
24.....	2	1 hour	"	+
436.....	2	2 hours	"	+ *
15.....	2	4½ "	"	+
493.....	2	8 "	"	+
5.....	2	12 "	"	+
380.....	2	19 "	"	+
841.....	2	24 "	1 colony	+
6.....	2	48 "	None	+ *

* The second transplant to agar showed a smaller number of colonies than the first, from which it may be concluded that these urines had a slight inhibitory effect on the colon bacillus.

B. prodigiosus did not reach the urine in any of the experiments, which was to be expected in view of the failure of a pathogen like *B. coli* to pass the renal membrane.

The right hand columns of the tables show that in a few cases the urine proved to be a poor culture medium for the injected organism. Thus the bactericidal effect of some urines on bacteria must always be kept in mind in experimental work of this nature, but that it plays as large a part as Jahn claimed does not appear to be the case. In a few instances the organisms failed to withstand exposure to the urine, but in the majority of cases they not only survived but multiplied rapidly.

The above results are not in accordance with any theory which holds that bacteria are "excreted" by the kidneys. For true excre-

tion to take place the bacteria should appear in the urine within a few minutes after introduction into the circulation and persist until they are practically all removed from the blood.

TABLE III

Bacteriological Examination of Urine at Varying Intervals after the Intravenous Injection of Bacteria in Rabbits

III. Bacillus Prodigiosus

Rabbit No.	Amount of suspension injected	Interval between injection and death of animal	Organisms in urine	Survival of organisms in inoculated urine
	cc.			
98.....	2	15 min.	None	+
99.....	2	30 "	"	+
95.....	2	1 hour	"	+
38.....	5	3 hours	"	+
96.....	5	5 "	"	+
34.....	5	8 "	"	+
2.....	5	12 "	"	+
381.....	5	18 "	"	Not done
1.....	5	24 "	"	—

EXAMINATION OF SECTIONS

Comparatively little attention has been devoted in this work to the development of lesions in the kidney after the injection of bacteria into the blood stream, since this has been well described on previous occasions. My interest has been chiefly in the determination of the exact location of the organisms in the renal substance. For this purpose the careful study and interpretation of the sections of incubated kidneys have proved to be most interesting and instructive.

A few typical fields in these sections are reproduced in the photomicrographs. In order to understand them properly it will be necessary to take into consideration a few simple facts. Bacteria growing within a confined space will naturally form colonies that conform in shape more or less to that of the space. For instance, if they are growing within a hollow sphere they will tend to form an approximately globular colony; if they are in a tube they will form a cast of the tube, and so forth. The peculiarities of the particular organism will also play some part. Thus a motile organism growing in a tube

will extend for some distance along the tube, while a staphylococcus will remain more definitely localized. Other factors also enter, such as the suitability of the nutriment, compactness of the tissues, and so on. For the sake of comparison, a section of kidney with the blood vessels injected with gelatin is shown in Figure 1.

Figure 2 illustrates a typical colony of *B. coli* growing within the capillary loops of a glomerulus. The well rounded outline of the bacterial clumps is quite characteristic. The delimiting walls of the colony, though not apparent in the photograph, are readily seen under the microscope. The shape of the colony alone, however, is sufficient evidence that the bacilli are lying within the capillaries of the tuft. It is only reasonable to conclude, then, that the bacillus from which this colony originated lay *within* a capillary at the time of death. In contrast, if the organism had penetrated to the capsular space, the resulting colony would appear on section in the shape of a ring or crescent. *Such a colony was never seen in any of the experiments with B. coli or B. prodigiosus.*

A colony of colon bacilli growing between tubules is seen in the next photograph. Theoretically, there are three possible sites for such a colony: (1) an intertubular capillary, (2) a lymphatic vessel, and (3) the tissue spaces. The first is almost certainly the probable one, but there is no way of proving it, since the containing wall, being so thin, cannot be positively identified. One thing is certain, though, and that is that the organisms are *not* within the lumen of one of the tubules, which are, of course, very thick-walled.

There is no doubt when a colony such as is shown in Figure 4 is encountered. Here the bacilli are seen growing among the red cells in a blood vessel.

The difficulty of determining in just what type of vessel the organisms lie is exemplified by a colony in the medulla, Figure 5. It is quite plain that the bacteria are growing within some sort of thin-walled tube, for the latter is situated alongside some tubules that are being compressed. One might argue that in this part of the kidney such a colony might be within the thin-walled portion of a loop of Henle. If that were the case, one would expect to find colonies further on in the course of the nephron, in a distal convoluted tubule or collecting tubule; such colonies were not seen, however.

The appearance of the colonies of staphylococci is much the same as of the colonies of colon bacilli. Because of the non-motility of the

cocci the colonies tend to be more or less spherical, whereas the motile bacilli often progress for some distance along the capillaries. This is well shown in Figure 6, where only about 3 capillary loops are filled, compared to the 8 to 10 occupied by the bacilli in Figure 2. The shape of the clumps of cocci makes it certain that the growth took place *within* the blood vessels and not in the space between the two layers of Bowman's capsule. Figure 7 shows a colony of cocci as it appears in the medulla. If it had grown in one of the large collecting tubules, such as surround it, there would have been no difficulty in recognizing the fact.

The colonies of *B. prodigiosus* are very similar to those of the colon bacillus.

The photomicrographs so far described were all taken from sections of kidneys removed within the first hour after the injection of the bacteria. In examining a large number of stained sections one occasionally comes across a colony, the exact position of which it is difficult to analyze, due to its large size, distortion of neighboring structures, and so on. But it may be reiterated here that in none of the *B. coli* or *B. prodigiosus* experiments were bacteria seen growing *within* the lumen of what could be definitely identified as part of the tubular system. In order to visualize such colonies it is necessary to examine kidneys from the experiments with staphylococci, where definite lesions have been established. Figure 8, from Rabbit 462, which was killed 48 hours after the injection of *Staphylococcus aureus*, shows clearly two colonies lying within the lumina of tubules. During the incubation of the kidneys the tissues undergo considerable autolysis; this renders somewhat indistinct the cytological detail, so that at times it is not easy to tell to what part of the tubular system a particular portion belongs. There can be no doubt, however, that these colonies lie within portions of the convoluted tubules. It is not possible to say how much of each colony was present at the time of death and how much was due to growth in the incubator. Apart from other considerations, such a figure is exceedingly useful in that it furnishes a guide to the appearance of colonies of organisms within the tubules.

An interesting study in contrasts is presented in Figure 9. This, too, is from Rabbit 462. It shows an intense inflammatory reaction, consisting of a central zone of necrotic tissue and a ring of polymorphonuclear leukocytes — a developing abscess. Five malpighian

corpuscles are seen in the central area, *A-E*. In one, *A*, the cocci have burst through the walls of the tufts and are growing all through the cavity. *F*, a tubule containing organisms, probably originates in *B*. Particularly noteworthy is the corpuscle *E*, containing clumps of organisms with smooth, rounded outlines; this glomerulus should be compared with those shown in Figure 2 and Figure 6. It is apparent that in spite of the fact that this glomerulus must have been severely damaged, situated as it was in the middle of the abscess, the walls have not yet given way, although there is a suspicion of a rupture at *G*. Such a picture emphasizes the strength of the endothelial membrane, and suggests the thought that if the capillaries (or the visceral layer of Bowman's capsule) were able to hold out against the cocci for 48 hours during life and 20 hours in the incubator, it is not likely that the normal capillary endothelium and the renal epithelium covering it would permit the passage into the capsular space of one or two cocci within a few minutes after their injection into the blood stream.

This method of locating bacteria in the kidney would appear at first sight to be an infallible one, but it must be confessed that there is one, at least, possible source of error. As a control measure it is necessary to know whether or not the organisms would multiply postmortem and so show up by this method of investigation if they should manage to get into the lumen of the *normal* renal corpuscle or tubule. One cannot adduce such a picture as Figure 8 as a solution, because in that case the kidney had been severely damaged by the infection and many of the abscesses had ruptured into the tubules, permitting the presence of such abnormal constituents as albumin and blood, and making available a better food supply for the organisms than that of the fluid present in the normal tubules. Nor can one assume that because the bacteria grew well in the bladder urine in most cases (see tables) they would necessarily thrive in the tubules, for the fluid in the first part of the nephron is of quite a different character from the urine that issues from the papillary ducts into the pelvis of the kidney. The glomerular filtrate undergoes great changes in salt content, volume and pH as it passes through the tubules, changes that affect materially its suitability as a culture medium.

In an attempt to learn whether or not the organisms would grow in the normal tubules, saline suspensions of *Staphylococcus aureus*

and *B. prodigiosus* were injected up the ureters of a pair of kidneys freshly extirpated from the body of a rabbit. This manoeuvre failed, either because the bacteria did not grow in the tubules, or because they never got that far. The latter is the more likely explanation — it is a well known fact that it is very difficult to get substances into the interior of the kidney by injection through the ureter.

THE SHIFTING OF SMALL PARTICLES IN HISTOLOGICAL SECTIONS

Several investigators, more particularly those who claim that the normal kidney excretes bacteria, have described finding the organisms actually lying in the capsular space and in the lumens of the tubules on microscopic examination. For instance, Schweizer,³ after pointing out how few organisms are to be found in sections, says: "I found the microorganisms to be most abundant by far in the glomeruli and in the space between them and Bowman's capsule. Then I found them fairly frequently in the blood vessels and in the lumen of the first part of the convoluted tubules. More rarely I saw them in the perivascular connective tissue. Only once could I with certainty demonstrate a bacillus between two epithelial cells of a convoluted tubule. Despite zealous searching I have never been able to find a coccus or a bacillus in the cells themselves."

Again, von Klecki⁶ says: "The bacteria could be demonstrated microscopically by thionin staining. For the most part only single organisms were encountered — in the glomerular loops, in the capsular space of the glomeruli, in the lumen of the first portion of the convoluted tubules, and mostly between the vessels accompanying the straight tubules; only once was a bacillus found between two epithelial cells of a convoluted tubule. Thus the glomeruli constitute the chief pathway for the excretion of bacteria through the kidneys: The bacteria penetrate the vessel wall of the glomerular loops, reach the capsular space, then the convoluted tubules and are washed out with the urinary flow."

Vincenzi,¹⁹ too, observed a few individual bacilli in the space bounded by Bowman's capsule as well as in the glomerulus. "After this discovery," he states, "it was clear to me that the excretion of the organisms injected into the blood-stream had taken place through the glomerulus." Rolly²⁰ did not see any bacteria in the sections, they were so few in number.

These observations, of course, fit in very nicely with a theory which holds that the normal kidney can pass bacteria, and it would seem impossible to reconcile such findings with the views of those who believe that the organisms do not get through to the urine in the first few hours after injection, and that they appear in the urine only after the renal tissues have been injured. We believe that there is a possible explanation, which depends on the fact that in the preparation of stained sections small portions of tissue are often displaced slightly from their original site. For example, on the edge of practically every paraffin section one may see small fragments of tissue, a single red cell or leukocyte, apparently floating in space — obviously detached at some stage in the preparation of the section. There is no reason why bacteria in the interior of sections should not be similarly affected, and that this does occur is shown in Figure 10, which shows the same glomerulus as in Figure 6 under a higher magnification. Note the cocci lying within the capsular space; any observer, seeing one or two such organisms would say, and with perfect justification, that they were lying between the parietal and visceral layers of Bowman's capsule and definitely outside the vascular system. But the shape of the colony makes it practically a certainty that the bacteria had grown within the capillaries and that before fixation they were all confined within the capillary loops. At some stage in the cutting and staining of the section, however, a few of the cocci were dislodged from the main mass. This movement, small though it may be, is of tremendous importance in a problem of this kind, for it means the difference between an organism lying within a capillary and within the lumen of Bowman's capsule. The same phenomenon is shown in Figure 11, which shows a colony of cocci in the medulla. Again, the shape of the colony indicates that the organisms grew out in a tubular structure, but the displacement of a few of the cocci is plainly apparent at the point indicated by the arrow.

This explanation would be valueless if each section contained great numbers of organisms, but such is not the case. Everyone who has attempted the search for the individual bacteria has commented on the surprisingly small number to be found, even though enormous numbers were injected. Indeed, this rapid disappearance of the organisms from the blood stream is most graphically shown in a series of incubated kidneys. Ten minutes after the injection a

cross-section of the kidney is studded with colonies, up to 150 in number. This number rapidly decreases, depending on the number and type of organism injected, until at 8 hours a similar cross-section shows only one or two colonies. It is now an accepted fact that this primary sterilization of the blood stream is performed mainly by the cells of the so-called reticulo-endothelial system.

It was noted that the shifting of a few bacteria from the main mass was nearly always in the same direction in each section. At just which step in the preparation of the sections this displacement takes place it is not possible to say definitely at present. A few experiments carried out with one paraffin block of tissue cut in different directions seemed to indicate that during sectioning the microtome knife would push a few of the bacteria away from their original resting place for a short distance. Another possibility is that minute portions of the tissue might float around in the Canada balsam before the latter has hardened. At any rate, these sections show that small particles of matter like bacteria can and do become displaced to such an extent as to lead to errors in deciding their exact situation.

DISCUSSION

It will readily be apparent from what has been said above that there is more to this problem than the mere injection of bacteria into the blood and the subsequent determination of their presence in the urine. The bacteriological method of examining the urine is almost too subtle, particularly when liquid culture media are used, for, obviously, it does not distinguish between organisms that have passed through the kidneys and any that may have reached the urine because of faulty technique. For this reason such procedures as those employed by Biedl and Kraus and others, who inserted canulae into the ureters in order to obtain the urine, are always open to question.

Not only do the results obtained in this work show that the normal kidney is not permeable to bacteria, but they also tend to indicate that the damage to the renal membranes must be of considerable degree before the organisms can pass through. More evidence in favor of this view is found in the results of a few experiments, not recorded in the tables, in which bacteria were injected into animals whose urine contained considerable albumin. The time of appear-

ance of the bacteria in the urine did not differ from those cases in which the urine was albumin-free. It would seem that organisms can penetrate into the urine through lesions that they themselves have caused, but do not necessarily pass through more readily if the kidney is already damaged by some other agent. Other workers have noticed the same phenomenon. Wyssokowicz ²¹ first induced acute nephrosis in animals by injecting such irritants as ammonium bichromate, cantharides, and so on, and, after a few days, injected the bacteria. In spite of the damage to the renal parenchyma, as shown by the presence of albumin and casts in the urine, the organisms were not excreted by the kidneys. Similarly, Helmholtz and Bowers ¹⁵ found that the presence of experimentally produced acute staphylococcic lesions in the kidneys did not predispose to the rapid passage of colon bacilli into the urine.

Although this investigation is intended to be mainly an experimental study, it may not be amiss to refer to one or two points that are of clinical interest. Probably the most important practical application of this problem is in renal tuberculosis. It has been known for some time that there are certain patients with tuberculosis in parts of the body other than the kidney whose urine contains tubercle bacilli, as shown by guinea pig inoculation. This occurs in the complete absence of symptoms referable to the urinary tract — “silent” bacilluria. Moreover, when these cases come to autopsy, not a few of them show absolutely nothing on ordinary gross and microscopic examination of the kidneys. The knowledge of these facts has had considerable to do with “confirming,” on the clinical side, the theory that the normal kidneys can pass bacteria.

Closer examination of all the circumstances throws considerable doubt on this claim. Much of the misunderstanding has arisen because tuberculosis of the kidney is usually considered in terms of gross lesions, principally of two varieties: the large caseating type, and that caused by miliary dissemination. But a point often overlooked is that miliary tuberculosis may be of all degrees, from an extremely mild, clinically unrecognizable form to one in which the dissemination is heavy and widespread, causing profound systemic reaction and ending inevitably in death. In other words, the number of tubercle bacilli that gain access to the blood stream may vary from a few to countless myriads. Hence it is easy to conceive of a light shower of bacilli resulting in a few very small lesions in the

kidneys, which discharge bacilli into the urine for a time and then heal, all without symptoms and without evidence at postmortem. This conception is supported by a study made a few years ago in this laboratory by Shipley, who examined the kidneys in some cases of tubercle bacilluria. The patients had complained of no urinary symptoms, the only signs of renal disease being the presence of the bacilli and a few pus cells in the urine. At autopsy no lesions were evident either in the gross or in the usual microscopic examination of a few blocks of tissue. It was only after serial section of considerable portions of the kidneys was performed that definite microscopic tubercles were found, which were quite sufficient to account for the bacilluria.

SUMMARY AND CONCLUSIONS

1. *Staphylococcus aureus* injected into the circulation of normal rabbits did not appear in the urine until between 8 and 12 hours later.

2. *B. coli* was absent from the urine even up to 48 hours after injection.

3. *B. prodigiosus* failed to reach the urine up to 24 hours after injection.

4. The examination of ordinary paraffin sections to determine the exact site of a few individual bacteria is not entirely reliable.

5. The method of incubating the kidney before fixation and staining not only facilitated greatly the finding of the bacteria, but permitted a more dependable analysis of the location of the organisms from which the colonies had developed.

6. By means of such examination it was seen that the bacteria never reached the capsular space about the glomeruli or the lumens of the tubules in the first few hours after injection, hence were not excreted. The probability is that they did not get beyond the vascular system until they had damaged the walls of the latter.

It is a pleasure to acknowledge the many kindnesses of Prof. Oskar Klotz, in whose department this work was carried out. The author is also greatly indebted to Prof. W. L. Holman, Dr. E. J. Clifford, Mr. Walter Cowan and many others in the Banting Institute for their suggestions and assistance.

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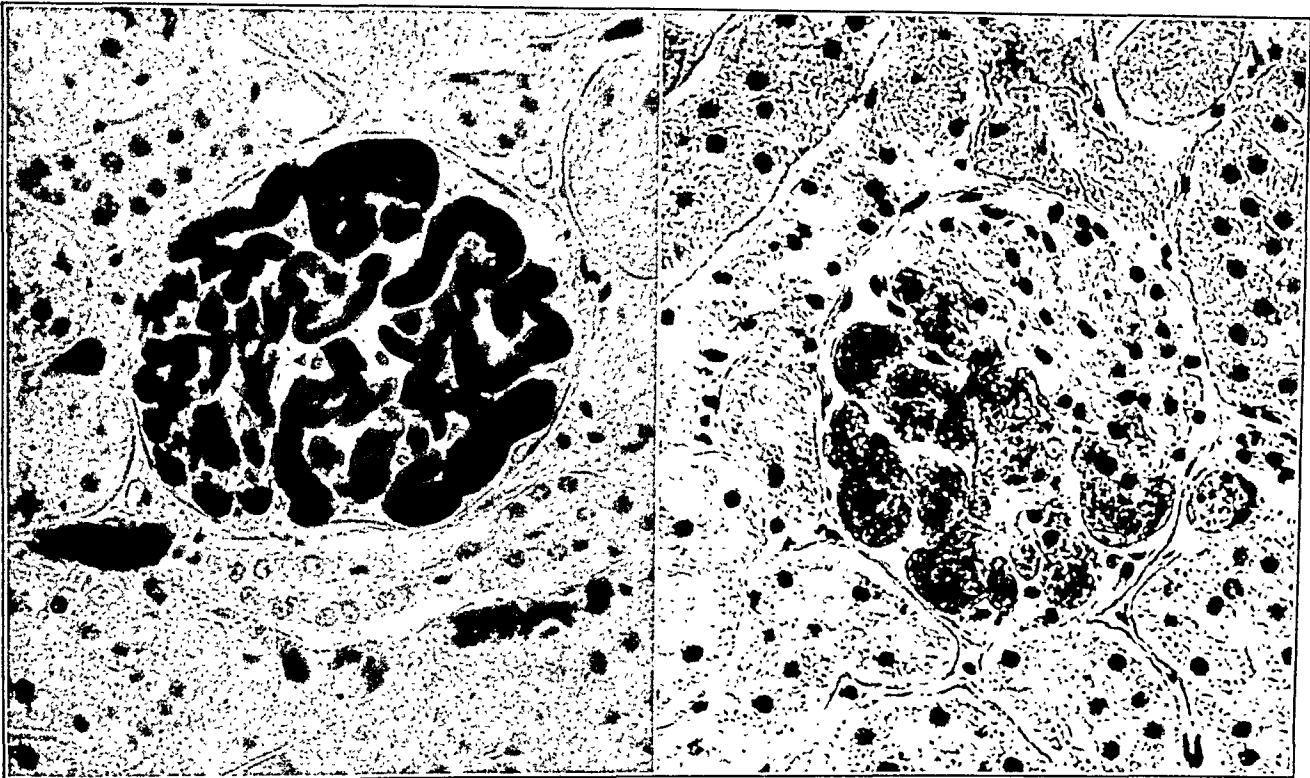
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DESCRIPTION OF PLATES

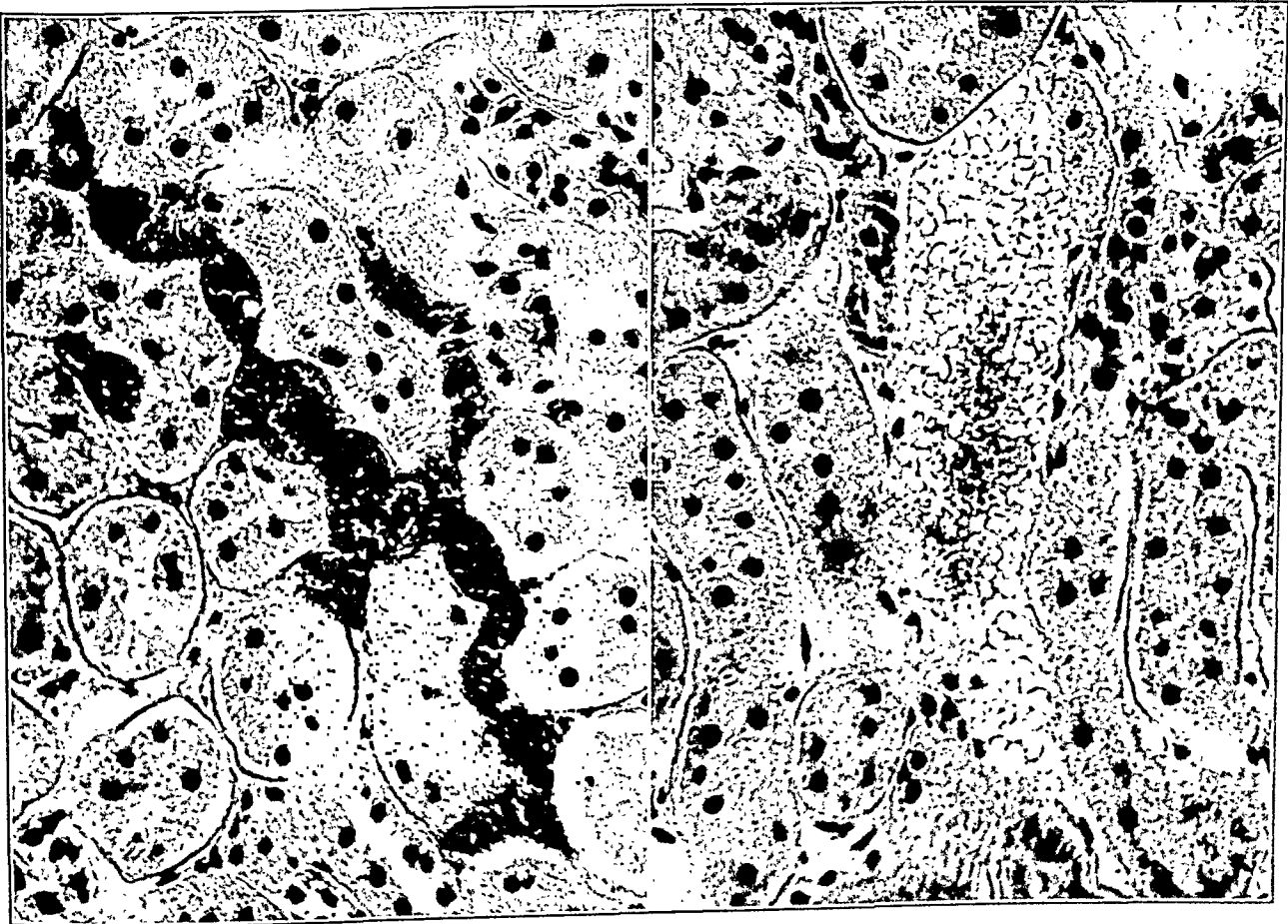
PLATE 95

- FIG. 1. A section of a cat's kidney with the blood vessels injected. $\times 410$ (approximately).
- FIG. 2. A colony of *B. coli* in a glomerulus. $\times 410$.
- FIG. 3. A colony of *B. coli* between convoluted tubules. $\times 410$.
- FIG. 4. A colony of colon bacilli within a blood vessel. $\times 410$.



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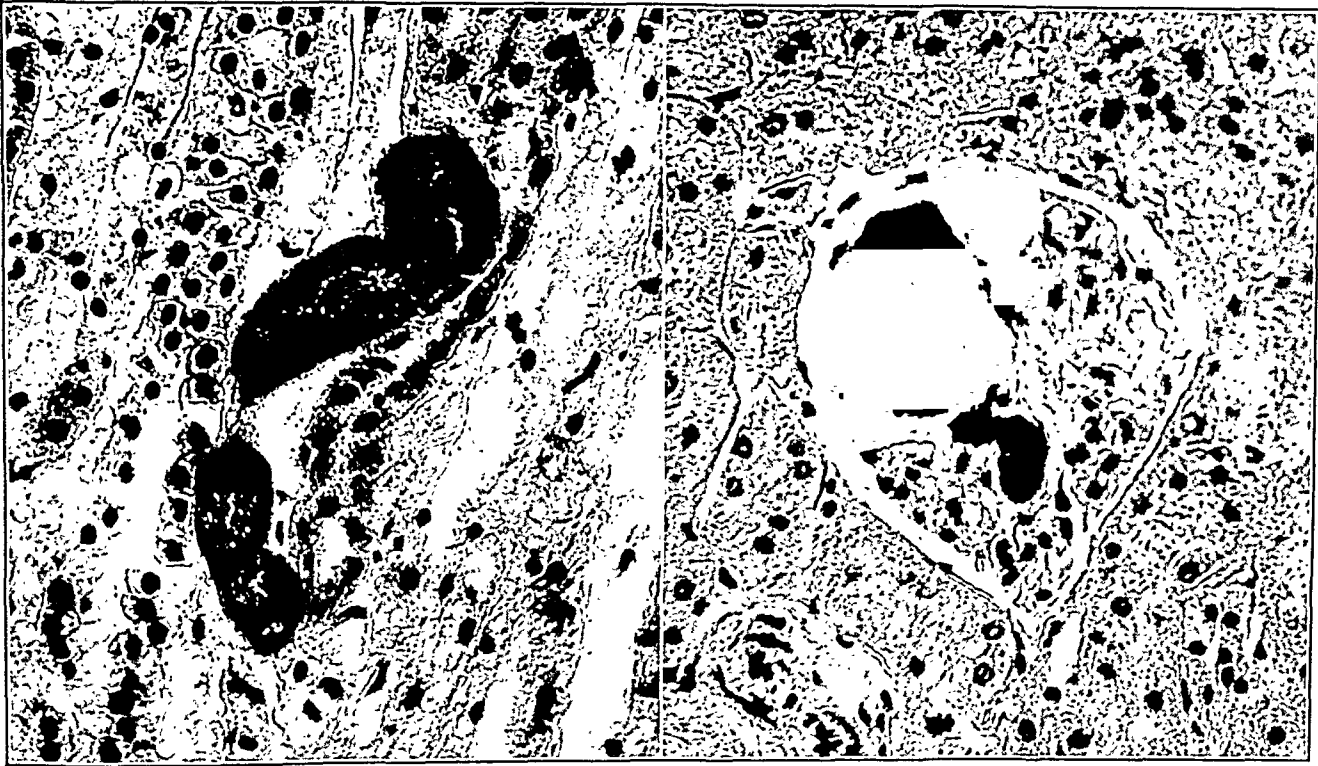
PLATE 96

FIG. 5. A colony of *B. coli* in the medulla. $\times 410$.

FIG. 6. A colony of staphylococci in a glomerulus. $\times 410$.

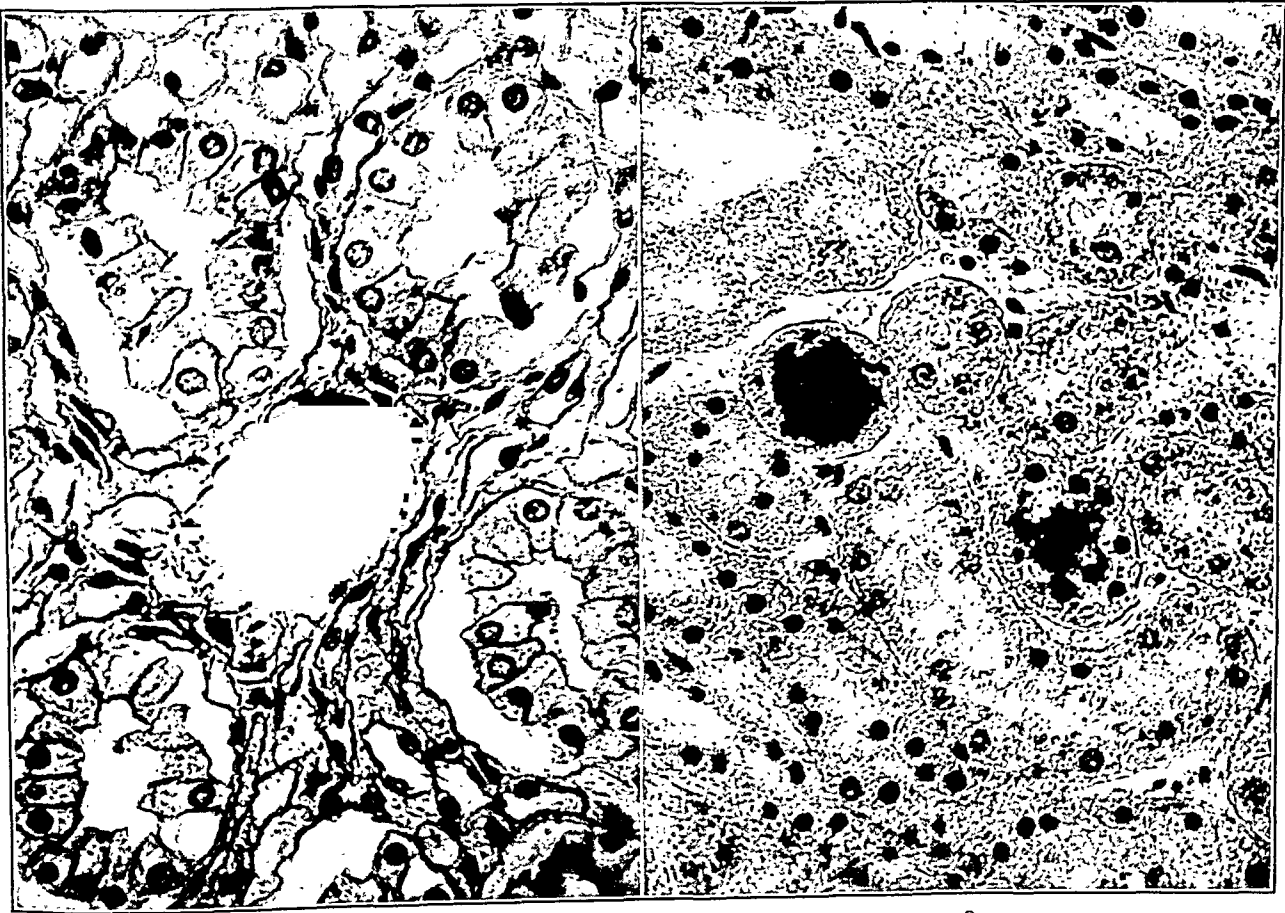
FIG. 7. A colony of staphylococci in the medulla. $\times 410$.

FIG. 8. Staphylococci within the lumens of tubules. $\times 410$.



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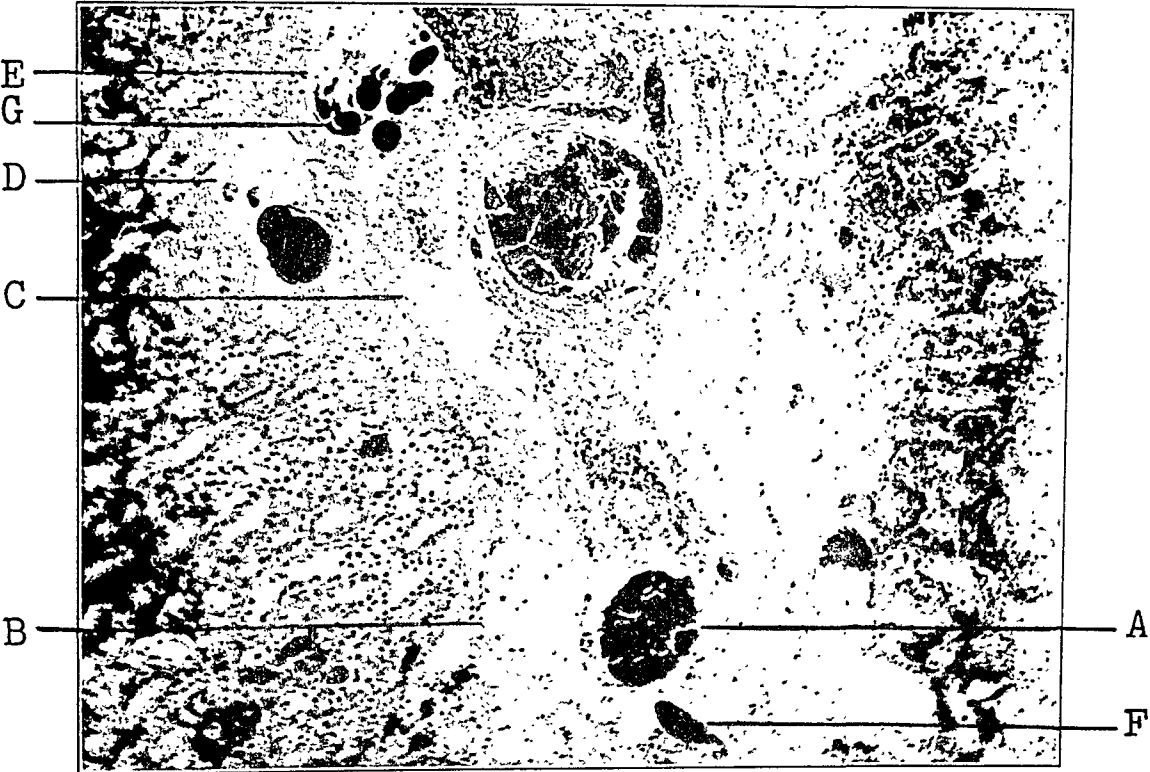
PLATE 97

FIG. 9. A developing abscess, due to *Staphylococcus aureus*. $\times 100$.

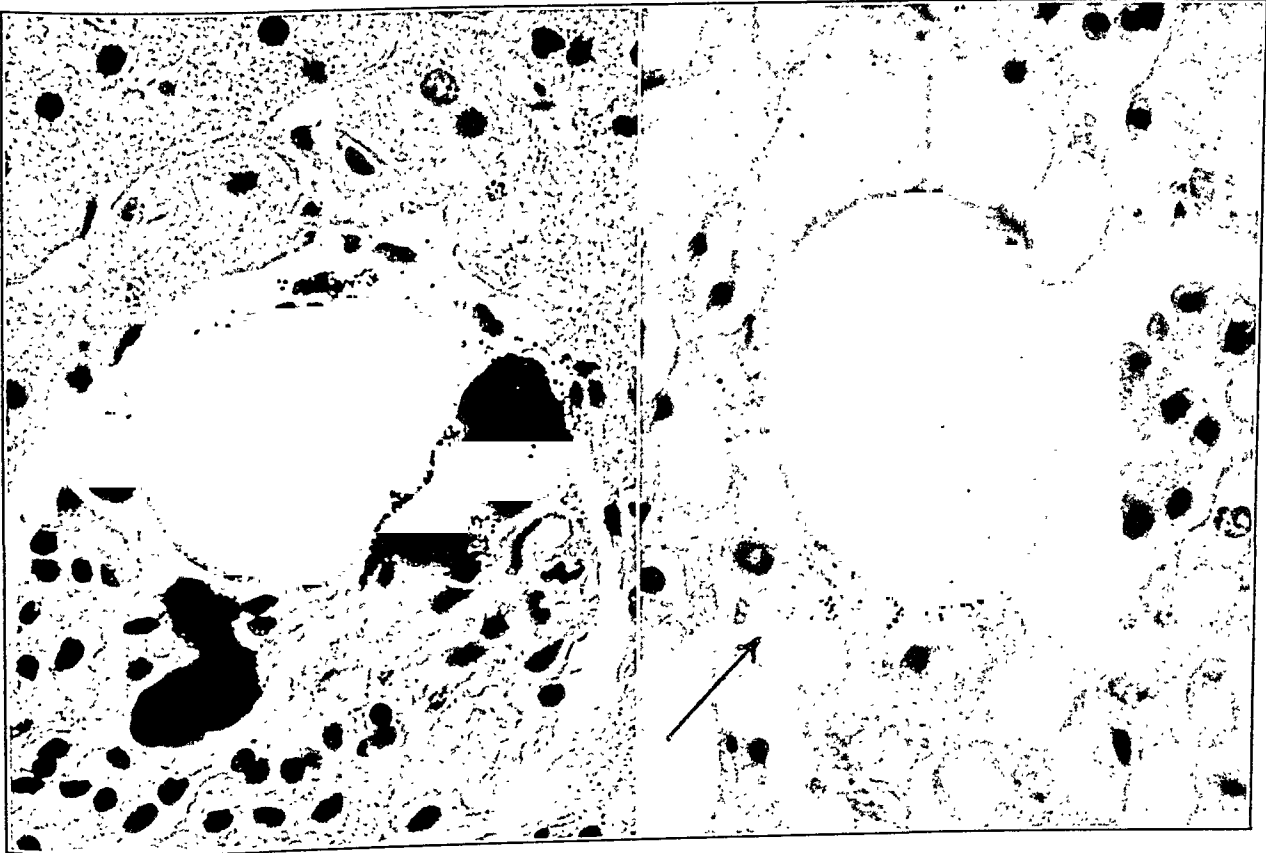
FIG. 10. Part of the same glomerulus shown in Fig. 6, to illustrate the shifting of bacteria in sections. $\times 900$.

FIG. 11. A colony of staphylococci in the medulla. $\times 900$.

The left and upper borders of the colony are not in focus in the photograph, but are seen to be quite sharp and well defined on direct examination with the microscope.



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THE MICROINCINERATION OF HERPETIC INTRANUCLEAR INCLUSIONS *

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In view of the frequent association of inclusion bodies with virus infections any knowledge that can be accumulated as to the nature of these bodies will be of value in the investigation of the etiological agents. With this in view a study of the inorganic residue of the inclusion bodies associated with the virus of herpes simplex was undertaken. The technique of microincineration as first devised by Policard ¹ and later modified by Scott ² was employed.

Scott ³ in investigating the ash content of the nuclear inclusions of the submaxillary gland disease of guinea pigs found that they left little or no ash after incineration. Cowdry ⁴ obtained similar results in his investigation of the nuclear inclusions produced by the virus of yellow fever. On the other hand, Covell and Danks ⁵ observed an abundant, compact ash content in the cytoplasmic inclusions of rabies. Danks ⁶ found a definite ash content in the residue of incinerated Borrel bodies of fowl-pox, also a cytoplasmic inclusion. These are the only inclusion bodies so far examined by the technique of microincineration, which leaves open the interesting question as to whether or not the study of the remaining inclusions will disclose a tendency for nuclear inclusions to show a relative absence of ash and for cytoplasmic inclusions to exhibit very much ash.

MATERIAL AND METHODS

The herpetic virus † was injected into rabbits under ether anesthesia by the intracerebral route in the usual way. The animals were sacrificed at various stages in the resultant reaction. Portions of their brains were immediately removed and fixed for 24 hours in a mixture of 9 parts of absolute alcohol and 1 part of neutral formalin. They were then passed through 4 or 5 changes of absolute alcohol at

* Aided by a grant from the Rockefeller Foundation for research in virus diseases.

† Obtained through the courtesy of Dr. T. M. Rivers.

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intervals of $1\frac{1}{2}$ to 2 hours, placed in a solution of equal parts absolute alcohol and xylol for $\frac{1}{2}$ hour, cleared in xylol for 12 hours, and embedded in paraffin.

Serial sections, 3 microns thick, were made from the left cerebral cortex near the site of injection. Adjacent sections were alternated on two slides, one slide for staining and the other for microincineration. Those for incineration were mounted with absolute alcohol and placed in an electric quartz oven, the temperature of which was gradually increased over a period of 35 to 40 minutes to approximately 620° C. The slides were then cooled, covered with No. 0 coverslips and sealed with paraffin. The control sections were mounted with egg albumin (3 drops of a mixture of equal parts egg albumin and glycerin in 50 cc. of water) and stained with Giemsa's stain.

The microscopic equipment and illumination for examination of the incinerated sections were the same as used by Cowdry⁴ in the histochemical investigation of the intranuclear inclusions of yellow fever. For simultaneous examination of the corresponding stained sections a Spencer monocular microscope, objective 1.8 mm. N.A. 1.25, equipped with an Abbé condenser was used. An iris diaphragm and 10 x and 20 x oculars were employed in the study of both stained and incinerated sections.

OBSERVATIONS

It was apparent from the start that the general topography of the incinerated and stained slides was remarkably similar. However, the striking difference in the stained slide between the normal nuclei and those containing inclusions was not so marked in the incinerated slide. For this reason positive identification of the same cell in both slides was necessary. When the cellular ash on the incinerated slide could not be recognized as identical with the cell containing the inclusion observed in the stained slide, it was not considered.

Insistence on this positive identification necessitated study of inclusions that were near such conspicuous landmarks as arteries and veins. The use of sections 4 and 5 microns thick resulted in so changing the configuration of the field as to throw doubt on the identification of a single cell on the two slides. It is for this reason that sections were cut at 3 microns as specified. By the alternate use

of incinerated and control sections it was then feasible to locate the ash on the incinerated section just 3 microns beyond the level of the stained sections, making possible the observation of the same inclusion in both slides. In many instances a single nucleus containing an inclusion could be located in three consecutive sections, for example, in two stained sections and in the intervening incinerated section.

The accompanying plate has been divided into two parts, left and right, to facilitate comparison of sections of the same nuclei as they appear in stained and incinerated preparations. Thus on the left are shown drawings of inclusion-containing nuclei stained with Giemsa and on the right, with the corresponding numbers, are shown drawings of adjacent sections of the same nuclei after microincineration. Nuclei of approximately the same size were selected and grouped to illustrate the contrast between young and old inclusions (Lauda and Luger ⁷). Younger inclusions than shown here may be found, but because they contain considerable basophilic chromatin which is known to leave a definite ash it would be impossible in the presence of this chromatin to determine how much, if any, of the observed ash was due to inclusion material. Only those inclusions, therefore, that exhibited no visible basophilic chromatin in their substance were included. When the inclusions had progressed to the stage that the nuclear membranes were no longer intact it not only became difficult to identify them after incineration but it was felt that the conditions would scarcely be typical.

In order to conserve space on the plate the cytoplasm has been omitted. Figures 1-4 show nuclei containing typical young inclusions that exhibit a marked contrast to the more advanced inclusions (Figs. 5-12) characterized by an increase in the halo and diminution of inclusion content. It is obvious that the younger inclusions exhibit a more abundant ash; in fact a definite residue is often difficult to demonstrate in the old inclusions (Figs. 10 and 12).

A study of the stained sections reveals an absence of any structure resembling a nucleolus, the disintegration of which has been previously described (Goodpasture ⁸). Nucleoli of normal cells of the type studied have a definite ash content unmistakable in configuration; such a body is not observed in the incinerated inclusion-containing nucleus.

Concentrated at the nuclear membrane are the basophilic masses that many investigators believe represent the chromatin material seen in the body of the younger inclusions. On the incinerated slide these masses leave a compact white ash.

DISCUSSION

The striking finding of this investigation is that as the inclusion ages the inorganic content diminishes. If one assumes that the inclusion represents a cellular alteration due to a physical or chemical influence, the presence or absence of ash would merely represent the acquisition and retention of inorganic constituents of metabolism. This might easily be the result of a change in the permeability of the nuclear membrane. Such hypothetical alteration is consistent with the diminution of the ash in old inclusions of herpes simplex, for, as is well known, the nuclear membranes about the very old inclusions disappear. Another possible explanation for this phenomenon may lie in a conversion of the inorganic content from a comparatively non-dialyzable form in young inclusions to a highly dialyzable form as the inclusion ages. Lauda and Luger⁷ have demonstrated that the nuclear membrane undergoes a degeneration in herpes simplex, even to the extent of obliteration. Therefore, it would seem likely that the change in inorganic content is due to a disturbance of the function of the membrane.

As previously explained, it was deemed advisable to minimize a gross source of error by limiting the observations to nuclei containing no visible basophilic chromatin in the inclusion body. Even this precaution does not guarantee the absence of basophilic chromatin and it is quite likely that the heavier ash of the younger inclusions may be due to chromatin that has not migrated to the periphery of the nucleus.

The investigations of Scott³ and Cowdry,⁴ on the submaxillary gland disease of guinea pigs and on yellow fever respectively, have disclosed a slight to doubtful ash content for the nuclear inclusions concerned, while Covell and Danks⁵ and Danks,⁶ working in the same laboratory and using the same technique, investigated rabies and fowl-pox respectively and found an abundant ash residue for the cytoplasmic inclusions. The finding of little or no ash residue in the mature herpes simplex inclusions is consistent with the generaliza-

tion that the nuclear inclusions examined by microincineration differ from the cytoplasmic ones in this respect.

If one assumes that the inclusion contains the etiological agent, be it organismal or inanimate in nature, it must be granted, in the light of studies thus far made, that either the inorganic content of the etiological agent varies from the great amount found in cytoplasmic inclusions to practically *nil* in intranuclear inclusions, or that the changes in inorganic material are the results of cellular changes aroused by the presence of the etiological agent. On the other hand, the difference in the inorganic residue may have no relation whatever to the specific etiological agents, for these agents may be merely acting at the interface between the cell membrane and the surrounding tissue or between the nuclear membrane and the cytoplasm.

SUMMARY

Microincineration of herpetic intranuclear inclusions from the cerebral cortex of rabbits reveals the presence of considerable inorganic material in young full inclusions with a progressive decrease in amount as the inclusions develop. Mature inclusions are frequently devoid of any inorganic residue.

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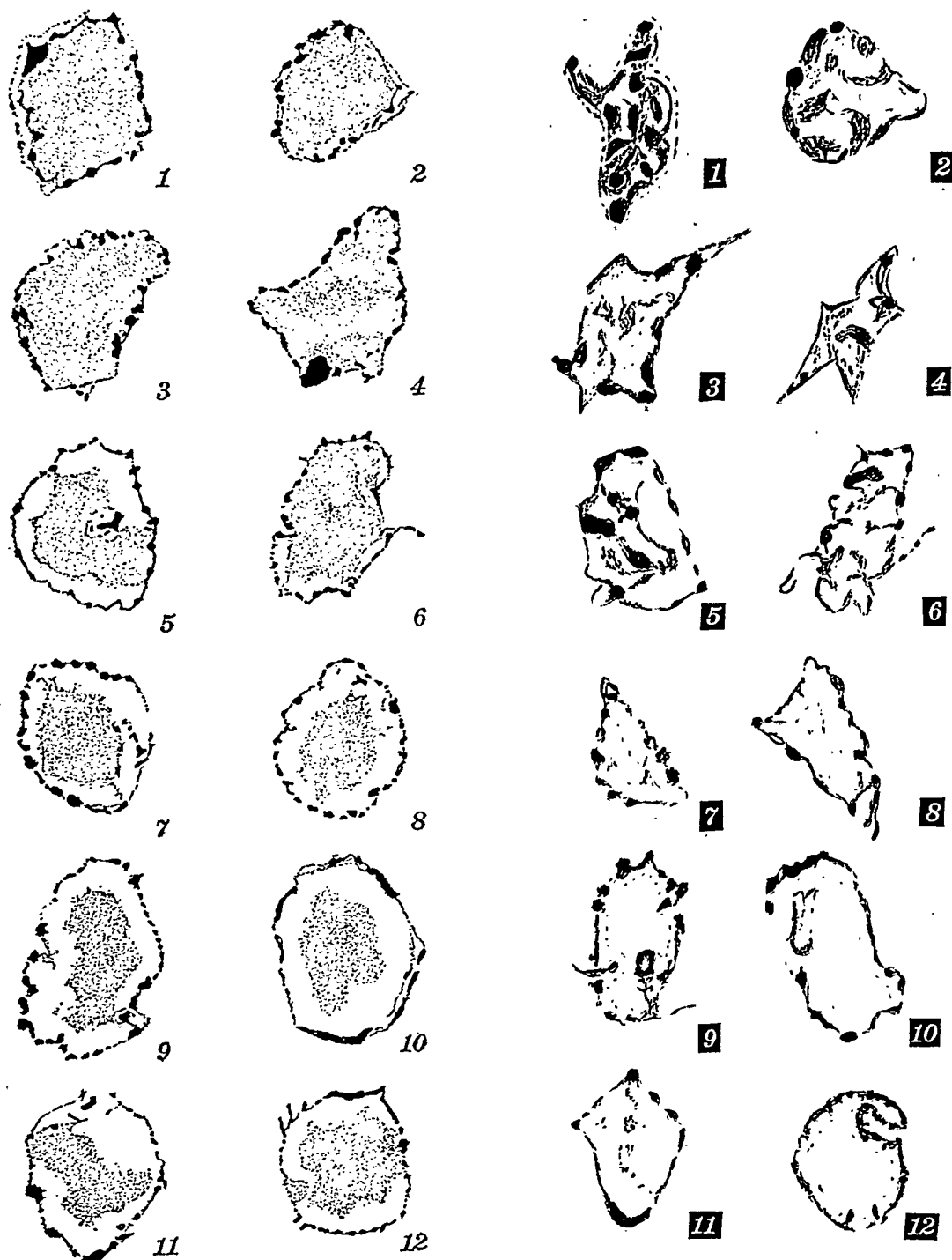
DESCRIPTION OF PLATE

PLATE 98

The left half of the plate contains drawings of cortical cells of rabbit made from stained sections and on the right half, numbered correspondingly, are drawings of the same nuclei made from adjacent 3 micron sections after micro-incineration. The cytoplasm has been omitted to save space.

FIGS. 1 to 4 inclusive. Drawings of stained and incinerated nuclei showing young inclusions of herpes simplex. Note the presence of ash throughout the nucleus.

FIGS. 5 to 12 inclusive. Drawings of stained and incinerated nuclei showing more advanced stages of inclusions of herpes simplex in varying degrees of advancement. Note the steady diminution in ash as the halo develops. The minute amounts of ash seen in Figs. 7 to 12 are typical of mature inclusions.



RARE FORM OF SACCULAR CARDIAC ANEURYSM WITH SPONTANEOUS RUPTURE *

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A not uncommon late complication of coronary arterial disease and cardiac infarction is ventricular aneurysm, which rarely ruptures except in the presence of reinfarction of the old scar or adjacent myocardium. Commonly such aneurysms develop only at the site of large and healed infarcts, are of considerable size and have a wide communication with the cavity of the chamber involved. Actually many of them represent merely a dilatation of the heart wall rather than aneurysm in the more restricted sense of the term.

Among a series of 40 cases of spontaneous rupture of the heart reported by us in a previous paper ¹ there was encountered one example of rhexis of a true saccular aneurysm communicating with the left ventricular cavity by only a small opening, so that the aneurysm lay over the external surface of the heart. In view of the unique character of the lesion, our failure to encounter an identical example among the exhaustive literature dealing with cardiac rupture, and because the length of the first communication necessitated only a brief consideration of individual instances, we have felt it advisable to make a more detailed account of this particular case

A similar aneurysm which, however, failed to rupture was described by Corvisart ² in 1797. It occurred in a negro 27 years of age who entered the hospital "in inexpressible anguish and anxiety; breathing was laborious and interrupted; he suffered little pain in the thorax, which besides, sounded well in its whole extent; he complained of feeling a violent pain both toward the region of the stomach and of the liver." Death occurred the following day. The heart was reported to be of natural size, "but the superior and lateral portion of the left ventricle was surmounted by a tumor almost as large as the heart itself." This tumor was of cartilaginous consistence but of the appearance of muscle, communicated with the ventricle by a small opening, and contained coagula.

* Received for publication May 22, 1933.

REPORT OF CASE

Clinical History: W. E. J., Male, aged 45, a lieutenant in the Portland fire department, died suddenly while driving his car. An autopsy was made at the direction of the coroner. Three months prior to death the man had consulted a physician because of severe pain in the stomach, radiating to the left arm, pain in the back and headache. On this occasion he appeared very ill and suffered intensely, requiring a half grain of morphine hypodermically for relief. The heart was irregular, the valve tones indistinct, the pulse feeble and the arteries were inelastic to palpation. The systolic blood pressure was recorded as 160 but there is no notation of the diastolic reading. A hasty urinalysis revealed a trace of albumin but nothing else of consequence. The past history was irrelevant except for loss of appetite and headache extending over a period of five years. In early life he drank to a moderate extent but for the past few years had indulged but very little. On the day of death he was on duty and feeling unusually well.

POSTMORTEM EXAMINATION *

For the sake of brevity only the more pertinent findings will be described.

Even before opening the pericardial sac the presence of hemorrhage was anticipated by the dark color and tenseness of the membrane. Upon incising the pericardium the sac was found to be filled with blackish clotted blood, obscuring from view the empty and contracted heart and forming a cast about it. When the clot was removed there was seen over the anterolateral aspect of the left ventricle an area of fibrous adhesions 7.8 by 4.5 cm. in extent binding the epicardium and pericardium. Similar bands obliterated the pericardial space over the surface of a mushroom-like mass situated well up toward the atrioventricular sulcus and between the anterior interventricular and circumflex branches of the left coronary artery (Fig. 1-A). Along the inferior border of this pouch and 1 cm. lateral to a line drawn through its center was a slit-like, ragged-edged opening 4 mm. in length. A probe passed into this defect led through the 1 mm. wall at the base of the aneurysm into its cavity which lay over the external surface of the left ventricle. On separating the patch of pericardial adhesions lying inferior to the aneurysm a layer of clotted blood was exposed in the fibrous tissue holding the pericardia together. Tracing the hemorrhage cephalad one found it leading to the rent in the inferior wall of the aneurysm mentioned previously. It would thus appear that rupture took place at this

* Path. No. 10,725, Group F, Case No. 35 (see Ref. 1).

point and that the escaping blood then dissected through the adhesions for some distance before finally breaking out into the unobliterated portion of the pericardial sac (Fig. 1-A). Further fibrous bands obliterated the pericardial space over the anterior and left aspects of the left atrium and the greater part of the posterior surface of the left ventricle, stopping abruptly 2 cm. from the interventricular septum posteriorly and 2.5 cm. from the apex inferiorly. In addition to dissection along the course already described the extravasated blood had also made its way along the base of the aneurysm over the surface of the left atrium as far as the point of entrance of the left pulmonary veins and into the space separating the root of the aorta and pulmonary artery, tearing adhesions wherever these existed along its path.

The aneurysm measured 4 by 4 by 3 cm., the last mentioned figure being the height above the surface of the heart. The wall varied between 0.1 and 0.7 cm. in thickness and appeared to consist solely of pericardium, fibrous connective tissue, epicardium and possibly endocardium, bound intimately together. The lining of the sac was generally smooth and whitish, except for linear grooves and ridges radiating outward in a fan-like fashion from an opening measuring 0.5 by 0.3 cm., located at the base of the cavity and leading directly through the myocardium into the left ventricular chamber. In several places the smooth and glistening white lining of the pouch was coated with a thin, yellowish brown film of fibrin; otherwise the aneurysm was empty.

An incision carried through the opening leading through the left ventricular wall disclosed patchy, whitish scar tissue in the myocardium immediately around the defect and for approximately 2.5 cm. outward into the muscle on all sides of it (Fig. 1-B). This was the only macroscopic lesion in any part of the heart muscle. Moreover, after sectioning it became evident that the fluted ridges around the margins of the opening into the aneurysmal sac were directly continuous with several greatly attenuated trabeculae carneae. Elsewhere these muscle bands were of average normal size except along the septal surface of the ventricle where there was some flattening and atrophy. The internal end of the communication between the ventricle and sac on its surface was situated 1.5 cm. from the attachment of the posterior mitral valve cusp to the mitral ring. The anterior and posterior walls of the left ventricle averaged

2 cm. in thickness, decreasing to 1.5 cm. at the point of the opening leading to the aneurysm and to 1 cm. at the apex of the chamber. The several valves of the heart and the endocardium of the different chambers displayed no evidences of disease.

The ostium of the right coronary artery appeared almost closed by sclerosis, while that of the left was greatly narrowed by the same process. Extending cephalad from the commissure separating the anterior and left posterior aortic leaflets were several shallow linear wrinkles in the intima but nothing further suggestive of syphilis was observed at any other point along the course of the aorta. Aside from the plaques obstructing the coronary orifices there were a number of small and beaded yellowish streaks in the intima of the sinuses of Valsalva. Similar plaques were scattered along the intima of the thoracic aorta.

The main left coronary artery was a large and freely patent vessel exhibiting only small atheromatous plaques. Its anterior interventricular branch was likewise of ample size, but in places the lumen was moderately obstructed by whitish sclerotic patches. The same was true for the circumflex division and the right coronary artery. The latter vessel did not extend for any appreciable distance past the margo acutis, and to compensate for this the left circumflex gave off a posterior interventricular branch which coursed toward the apex and formed an adequate anastomosis with the anterior descending artery. At the point of bifurcation of the left coronary there originated two good sized twigs which supplied the portion of the left ventricle where the aneurysm and myofibrosis were located. The smaller of these passed directly to the area of scarring about the opening leading through the ventricular wall, and macroscopically its lumen at one point seemed to be occluded by an old and organized thrombus. No fresh thrombi were discovered in any of the coronary vessels.

The heart, together with the adherent portion of the pericardium and ascending aorta, weighed 640 gm. The kidneys had a combined weight of 440 gm. The capsule of each stripped readily, revealing a faintly and finely granular surface. On section the cortex appeared to be of normal thickness and the rays and labyrinths were everywhere plainly outlined. The lungs, spleen and liver displayed nothing other than acute passive hyperemia.

MICROSCOPIC EXAMINATION

Heart: Sections from blocks cut to include the opening in the left ventricular wall and the adjacent myocardium for 2.5 cm. on either side of it reveal irregular patches of connective tissue interrupting the muscle substance (Fig. 1-B). For 5 to 7 mm. around the margins of the hole the tissue is almost wholly fibrous but becomes progressively less so farther away from the defect. Several of the trabeculae carneae in this region are reduced to dense scar tissue or show only occasional persistent muscle cells. Between two of these muscles small thrombi consisting of fibrin and leukocytes are discovered. Near the opening the fibrous connective tissue nuclei are larger and more numerous than elsewhere and yet are widely separated by an abundance of coarse and compact intercellular collagen, among which is a fair number of thin-walled and engorged blood vessels. More peripherally the vessels are larger and possess a definite muscularis. None are occluded by thrombi nor do they exhibit evidences of arteriosclerosis. Approaching the margin of the aneurysmal opening one finds the scar replacing the myocardium passing uninterruptedly from the sac on the external aspect of the heart along the defect to become continuous with the endocardium. At the junction of the scar and endocardium there is edema, engorgement of capillaries and hemorrhage by diapedesis. The connective tissue cells forming the immediate lining of the communication uniting the ventricular cavity and the aneurysm have very large and hyperchromatic nuclei whose long diameter lies parallel to the opening and at a right angle to the myocardial cells. In this location the tissue is compact rather than loose, possibly indicating a compression by the pulsation of the blood passing through the opening. A true endothelial lining is lacking but the inner surface is nevertheless smooth and not covered by thrombi. As in other parts of the scar there are moderate numbers of capillaries about which are lymphocytes, plasma cells and histiocytes.

In the wedge-shaped area between the epicardium and base of the aneurysm (see Fig. 1-B) there is a rich fibroblastic proliferation and the whole zone consists of these cells and histiocytes. The muscle cells adjacent to the epicardium are hypertrophic, as evidenced by their great size and large hyperchromatic nuclei. Quite generally the muscle fibers are separated by very loose and apparently edematous

connective tissue. A deposit of fibrin covers the epicardium along this angle.

Sections from a number of different parts of the aneurysm wall reveal the lining to be undulating, interrupted by occasional sharp clefts, and consisting of compressed fibrous connective tissue, at times coated with a thin layer of fibrin or blood platelets (Fig. 2). Nothing resembling normal endocardium is identified. Passing from within outward there is encountered first a zone of solid connective tissue with abundant intercellular substance, followed by loose edematous tissue of the same nature containing numerous but irregularly distributed capillaries distended with blood and sometimes surrounded by fresh hemorrhage. At least one of these vessels exhibits a definite rupture of its wall, indicating that in this instance at least the surrounding hemorrhage is due to rhexis. Next there is a very active fibroblastic zone displacing almost entirely the fat cells of the subepicardial region. The blood vessels here are distended and colared by numerous lymphocytes, plasma cells, histiocytes, a sparse sprinkling of neutrophilic polymorphonuclear leukocytes and multinucleated giant cells engorged with yellowish blood pigment. The pericardium and epicardium are so intimately adherent that it is practically impossible to distinguish one from the other. Two coronary arterioles included in the subepicardium display proliferation of the intimal cells with resultant narrowing of their lumens.

Still another section, coming from the vicinity of the rupture through the aneurysm wall, discloses extensive recent hemorrhage into the fibrous adhesions between epicardium and pericardium and probably represents another ramification of blood from the point of rupture. More inferiorly and toward the apex of the ventricle the hemorrhage spreads out extensively and diffusely, breaking up and widely separating the adhesions. By these routes the dissecting hemorrhage must eventually have reached the non-obiterated portion of the pericardial sac and led to fatal hemopericardium.

The main left anterior interventricular coronary artery is the seat of intimal atherosclerosis, which has produced eccentric narrowing of the lumen. The deeper and more external parts of the plaques are often the seat of fatty degeneration and contain dust-like particles of lime salt. There are no apparent breaks in the endothelium. Lying free within the lumen and only partially occluding it is a small

ante mortem clot consisting of fibrin and leukocytes. The media is thin and atrophic while the adventitia is unaltered.

Microsections from three different points along the course of the small artery originating at the bifurcation of the main left coronary artery and coursing directly toward the scar about the aneurysm opening disclose advanced atherosclerosis of the intima with pronounced narrowing and eccentric displacement of the lumen. Thrombosis is lacking, although a few scattered erythrocytes and leukocytes are present. The deeper parts of the various patches of sclerosis are fatty, containing fat-laden phagocytes and large plates of calcium, not infrequently encased by multinucleated foreign body giant cells. Also there are several endothelial-lined, blood-containing channels in the degenerated parts of the plaques, but nothing whatsoever to indicate the existence of an organized and recanalized thrombus. The media of this vessel is thin and in places infiltrated with lymphocytes and plasma cells, yet without evidence of necrosis or scarring such as might be anticipated in chronic syphilis. Similar but larger cell collections collar the adventitial vessels in which evidences of endarteritis are lacking.

Microscopically the right coronary artery displays the same atherosclerotic process present in the other arteries with the additional finding of atheromatous ulceration. Although there is stenosis the lumen is not thrombosed. Perivascular accumulations are lacking.

The histopathological changes in the aorta are quite the same as those described for the coronary vessels, namely, varying degrees of intimal atheroma with softening, collars of lymphocytes and plasma cells about the vasa vasorum of the media and adventitia, sometimes with minute flame-like scars but never obliterative endarteritis.

Kidneys: A microscopic study of these organs reveals occasional completely hyalinized glomeruli, hyperemia of all other glomerular tufts, and subintimal hyaline thickening of certain intertubular and afferent glomerular arterioles, leading in some instances to moderate stenosis.

Pathological Diagnoses: Arteriosclerotic narrowing of coronary arteries, especially pronounced in one branch of the left; patchy myofibrosis of left ventricle with formation of saccular aneurysm, spontaneous rupture of this aneurysm and fatal hemopericardium; focal

fibrous pericarditis; atherosclerosis of aorta; possible rheumatic arteritis and aortitis; hypertrophy of heart; slight arteriolosclerosis of kidneys; probable hypertensive cardiovascular renal disease; generalized acute passive hyperemia.

DISCUSSION

From the foregoing description it is clear that death was the result of spontaneous rupture of the thin and fibrous wall of a very unusual type of cardiac aneurysm and the pericardial adhesions about it. The mechanism by which the aneurysm was produced is less easily explained. Although meager, the clinical data suggest that on the one occasion, three months prior to death, when the man was seen by a physician he was suffering from cardiac symptoms referable to the heart and compatible with coronary thrombosis. The one other significant observation at this time was the systolic blood pressure of 160 recorded during the seizure. It is common knowledge that the blood pressure decreases after a coronary accident and it is probably not erroneous to postulate that prior to the attack of cardiac distress the tension was greater than 160 mm. Hg. In any event the man had hypertension during the seizure and it is unnecessary to prove that the blood pressure was ever greater. If we accept Bell and Clawson's³ criteria for the clinical and pathological diagnosis of essential hypertension this case becomes a clear-cut instance of hypertension, for not only was the blood pressure elevated beyond the normal limit of 150 mm. of mercury during life, but at autopsy there was found cardiac hypertrophy (640 gm.) without demonstrable cause other than possibly from pericardial adhesions and the characteristic renal arteriolar lesions of essential hypertension.

Granting that primary hypertension formed the basis of the vascular disease there yet remains the problem of genesis of the aneurysm. In the absence of demonstrable coronary thrombosis one must seek to explain the myofibrosis on other grounds. Slow and progressive ischemia of a small area of myocardium brought about by high grade atherosclerosis of the vessel supplying the myocardial scar may have led to disappearance of patches of heart muscle and finally replacement fibrosis. Less probably the scar may represent the end-result of focal rheumatic or syphilitic myocarditis. Definite proof of

the existence of either of these diseases is lacking but certain features about the perivascular aortic and coronary arterial lesions are more suggestive of the former.

When and how the aneurysm formed is a question to which we can give no definite answer. In view of the clinical history there is reason to believe that it may have had its inception three months prior to death either during or shortly after the attack of severe thoracic pain and cardiac embarrassment. It seems possible in the presence of hypertension there may have been sufficient increase of intracardiac pressure exerted over the small area of already existent fibrosis to cause stretching or dissection until the external surface of the ventricle was reached, after which instead of immediate rupture the scar became adherent to the pericardium and these structures continued to hold for a time before finally giving way. Still another possibility is that of cardiac infarction, partial rupture during the acute stage of necrosis, followed by the formation of pericardial adhesions and finally rupture.

Although ordinarily cardiac aneurysm develops along the whole extent of the scar following healing of an infarct we have seen at least one striking exception to the rule. This case (No. 8370) (Fig. 3) may possibly represent an earlier stage of saccular aneurysm than the one forming the basis for our communication, and if so serves to show that not all cardiac aneurysms demand a large area of scarring for their development. In the example just mentioned a portion of a massive healed infarct involving the anterior wall of the left ventricle and interventricular septum was separated from the main cicatrix by a zone of intact myocardium and in the small part there formed a little pouch near the apex and adjacent to the interventricular septum. The peculiar and unusual feature was the fact that on either border of the wholly fibrous wall of the sac the ventricular muscle was of normal structure and thickness so that it appeared as if the aneurysm had been punched out over only a small area. As in the first case (No. 10,725) the neck of the pouch was somewhat narrower than the sac itself and the aneurysm wall externally consisted solely of fibrous tissue and epicardium. What would have been the later history of this aneurysm had the individual survived for a longer time is conjectural, but it seems possible that it might later have ballooned out over the external aspect of the ventricle as in the

first case, although the presence of the tampon thrombus that filled the sac may conceivably have organized and precluded such a complication.

SUMMARY

A rare and almost unparalleled type of cardiac aneurysm situated over the external aspect of the left ventricle is described and the possible causes for its development are discussed.

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DESCRIPTION OF PLATES

PLATE 99

FIG. 1-A. Case No. 10,725. Drawing, actual size. Anterior aspect of heart showing position and appearance of saccular aneurysm after removing a portion of its wall.

a = aneurysm.

o = opening with fluted margins, leading to left ventricular cavity.

R = point where rupture began at base of aneurysm wall.

r = fresh dissecting hemorrhage in pericardial adhesions.

h = torn fibrous adhesions over epicardium.

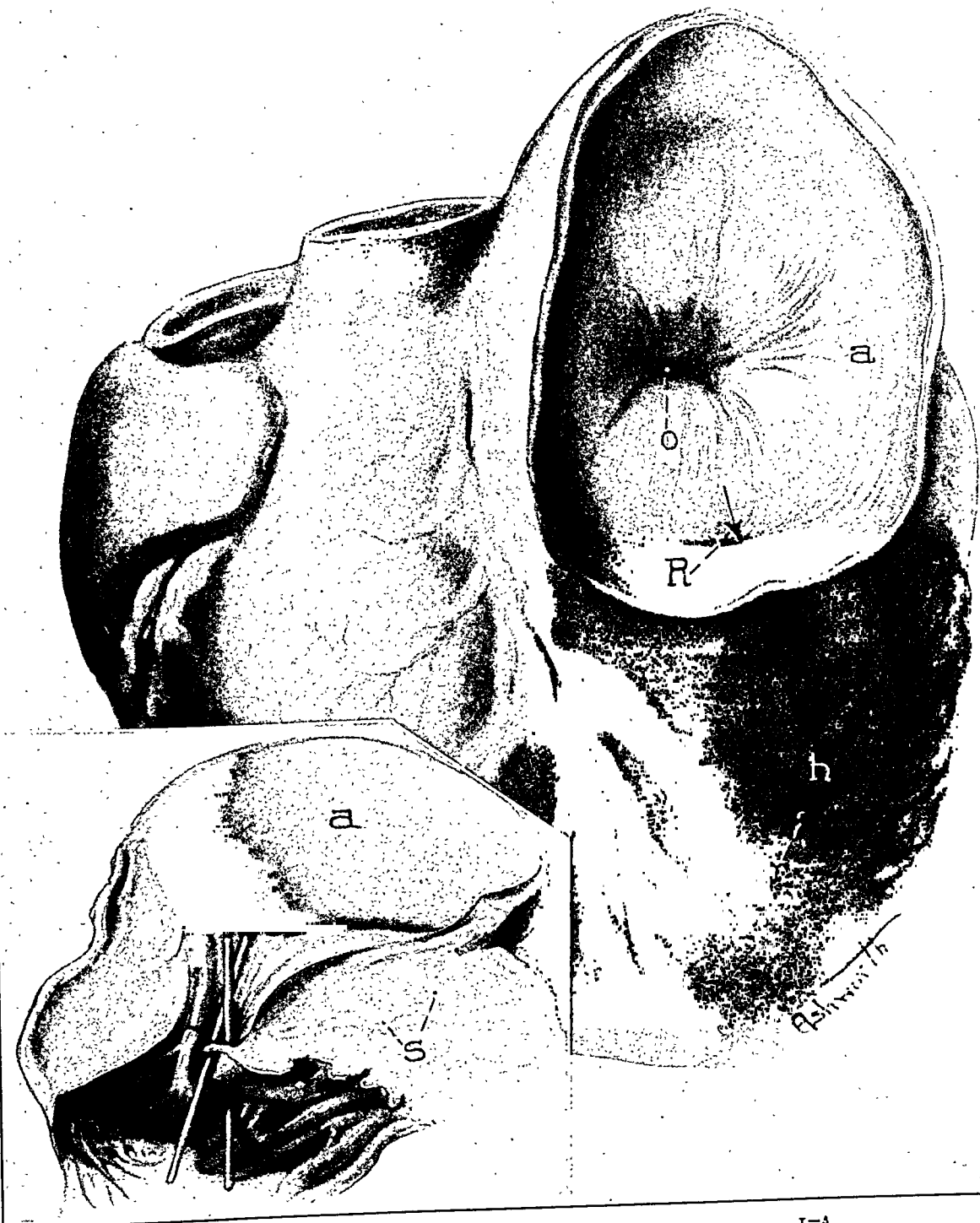
FIG. 1-B. Actual size drawing of aneurysm and left ventricular wall after hemisection of the sac to display the size and character of the defect and structure of adjacent heart muscle.

a = aneurysm.

s = patchy myofibrosis in vicinity of opening leading to aneurysm.

m.v. = posterior cusp of mitral valve.

Marker rods indicate ramifications of the channel between the atrophic trabeculae carneae and the main communication.



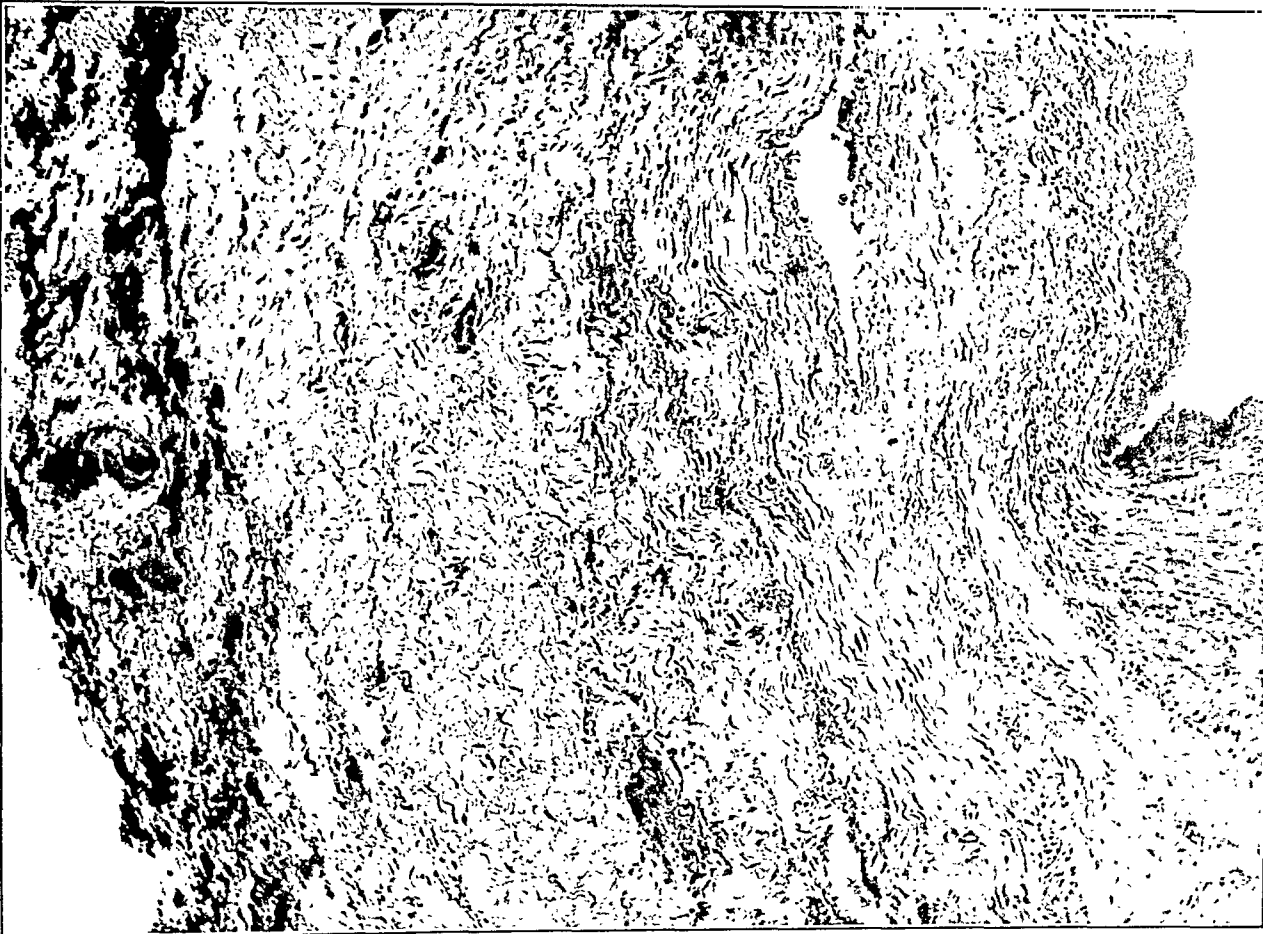
I-B

I-A

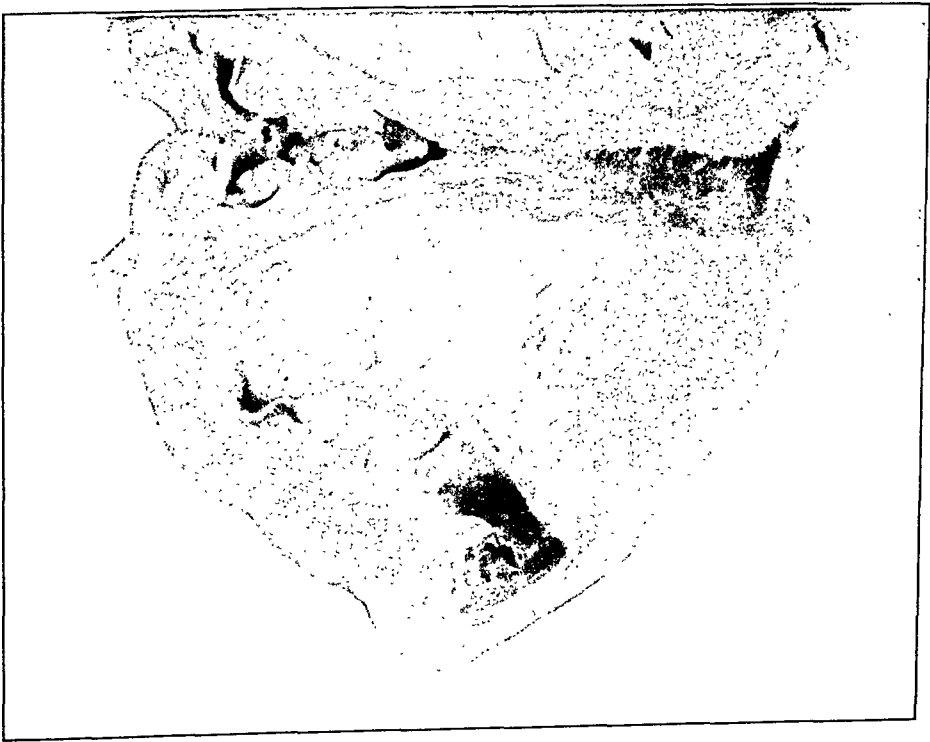
PLATE 100

FIG. 2. Low power photomicrograph showing entire thickness of cardiac aneurysm in case No. 10,725. To the right is seen a small portion of the undulating lining of the sac, at this point coated with fibrin. On the left border are torn vascular fibrous adhesions replacing the epicardium. All of the remainder of the wall consists of relatively young fibrous connective tissue in which a number of distended capillaries are visible. The complete absence of myocardium is evident.

FIG. 3. Case No. 8370. Photograph displaying septal surface of left ventricle. The interventricular septum (sectioned) and anterior wall of the ventricle are the seat of a healed infarct extending to the endocardium, which is greatly thickened and whitish. Near the apex is a small aneurysm surrounded by intact myocardium of normal thickness. Only the tamponing thrombus, thin fibrous wall of the sac and epicardium separate the cavity of the ventricle from the external surface of the heart.



2



3

ACTINOBACILLOSIS OF MAN *

REPORT OF A FATAL CASE

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In 1902 Lignières and Spitz,¹ working in the Argentine, identified the *Actinobacillus* as one of the etiological agents of so-called bovine actinomycosis. They proposed the designation "Actinophytose à actinobacille" for the disease, which by subsequent investigators has been called actinobacillosis.

Clinically and pathologically bovine actinomycosis and actinobacillosis have few distinguishing characteristics. The chief point of difference is situation. Actinomycosis usually involves the jaw bones as a rarefying osteitis with formation of sinuses, whereas actinobacillosis more often attacks the soft tissues of the mouth, pharynx, tongue and skin, with secondary involvement of the adjacent lymph nodes. Notwithstanding the clinical and pathological similarities of the diseases, the causal organisms are quite distinct, except in one feature. Both have the property of evolving aggregations in the tissues and exudates known as sulphur granules, about which radiating clubs are easily identified. On crushing the granules, however, the *Actinobacillus* yields only small Gram-negative bacillary forms, whereas *Actinomyces* reveals the distinctive branched Gram-positive filaments. Lignières and Spitz found the *Actinobacillus* capable of producing an experimental disease of cattle identical with the natural infection. It possessed a variable virulence for guinea pigs and rabbits.

The conclusions of Lignières and Spitz have been confirmed repeatedly by investigators working on the continent of Europe, the British Isles and Canada. Recently Thompson^{2,3} reviewed the subject, and reported for the first time the existence of actinobacillosis as a common malady of cattle in the United States. He concluded: "Actinobacillosis is common among cattle in the United States. It would seem that the condition here is similar to that in other coun-

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tries where the greater percentage of so-called bovine actinomycosis is due to *Actinobacillus lignièresi*." For more complete consideration of the subject reference may be made to Thompson's paper.

Thompson and Willius⁴ in 1932 reported the identification of *Actinobacillus* bacteremia in man. The patient was subsequently observed by Lawrence, Neuhauser and Howell⁵ and in a confirmatory, supplementary report it was stated by these observers that the illness had apparently terminated in recovery. Ravaut and Pinoy⁶ in 1911 described the occurrence of *Actinobacillus* meningitis. Their patient likewise recovered. The present case of *Actinobacillus* infection, the third in man to be reported, was fatal; consequently it is the first in which pathological studies have been made. The presentation of this case is our objective in this paper.

REPORT OF CASE

Clinical History: A man, aged 34 years, came to The Mayo Clinic Jan. 9, 1933. He had been employed as a clerk in a clothing store until a few months before, and more recently had been engaged in selling floor wax throughout southern Minnesota. He was born in southeastern Minnesota, where he always had resided. His health had been quite normal until the summer of 1932, when he began to lose weight gradually, for no apparent reason. Periods of anorexia were sometimes experienced. All bodily functions apparently had remained normal. He had not experienced excessive thirst or polyuria. There was no cough. On Dec. 25, 1932, he had no appetite, felt poorly and that evening retired early. On December 27 he became acutely ill and went to bed. The illness was considered by the patient to be influenza; it was characterized by malaise, weakness, fever to 102° F, and profuse perspiration. About Jan. 1, 1933, severe pain developed in the thorax posteriorly between the shoulders. Plasters were applied and on January 7 the pain entirely disappeared. The illness, however, was progressive; appetite failed, weight was lost more rapidly and weakness increased to the point of exhaustion. Fever continued, and on January 8 drowsiness and mild delirium supervened.

On the patient's admission to the clinic he appeared to be acutely ill and very weak. His temperature was 102° F, pulse 116 beats and respiration 32. Rather rapid loss of weight was evident (from 185 to 135 pounds). The skin was warm and moist. The eyes were rather prominent. The tongue was coated and the pharynx hyperemic. An enlarged cervical lymph node was palpable on the right side. A friction rub was noted in the right midclavicular line at the level of the fourth rib. Resonance was impaired at the bases of both lungs and moist râles were elicited at the base of the left lung. The heart was slightly enlarged, by percussion. The abdomen was distended and held tense; peristalsis was active. Roentgenograms of the thorax were interpreted as revealing extensive pneumonic consolidation at the base of the left lung, probably with fluid. A moderate degree of anemia was present. The concentration of hemoglobin was 69 per cent, erythrocytes numbered 3,510,000 and leukocytes 7000 in each cmm. of blood. The percentages of the various types of leukocytes were as

follows: polymorphonuclear neutrophils 77, lymphocytes 12 and monocytes 11, with changes in the leukocytes indicative of severe infection. Urinalysis revealed albumin, graded 2; sugar, graded 2; hyaline casts, graded 4, and an occasional erythrocyte; acetone was not present. Urea was present in the blood in essential normal concentration (44 mg. in each 100 cc. of blood), and sugar elevated (326 mg. in each 100 cc. of blood). Agglutination reactions for typhoid, paratyphoid and undulant fevers were negative. The Wassermann reaction of the blood was negative.

The patient was placed on a diabetic regimen and with use of insulin the blood sugar was brought to within normal limits. The fever increased to 103° F, and remained elevated, except for a rather sharp fall to 98° F on the morning of January 11, with subsequent rise to 102.8° F by noon of that day. The pulse and respirations remained rapid. On the evening of January 11 his breathing became more labored and respirations markedly accelerated. He was placed in an oxygen tent but failure was progressive and rapid. Death occurred at 12.17 A.M., Jan. 12, 1933.

At the time of death the diagnosis as to type of infection still was indeterminate. In many respects the illness resembled typhoid fever, and also influenzal pneumonia. The pneumonia, however, was atypical. The severity of the illness seemed to be out of all proportion to the physical or laboratory findings. The evidence of severe toxemia did not appear to be explained by the findings noted in the thorax, or by the diabetic state. Cultures of blood, which had been taken January 11, still revealed no growth of microorganisms when death occurred. The onset of symptoms of the fatal infection was probably about Dec. 25, 1932, and the preceding loss of weight was the result of diabetes. It is also probable that the presence of diabetes accentuated the infection and accounted for its severity, with rapidly fatal termination.

PATHOLOGICAL DATA

Throughout both lungs were multiple, firm, grayish nodules that measured from 2 to 5 mm. in diameter. The nodules were subpleural and were also within the substance of the lung. Nodules observed beneath the pleura were distinctly circumscribed and somewhat elevated. From some of them thick, yellowish pus exuded on section, whereas others were composed of grayish consolidation or caseous material. The spleen weighed 748 gm.; it was embedded in a mass of adhesions, and was composed largely of multiple, discrete, sharply circumscribed, grayish yellow lesions varying in size from 1 mm. to 1 cm. (Fig. 1). On section these lesions contained thick yellow pus or material of thick pasty consistence, which resembled

that of caseation necrosis. The liver weighed 2600 gm. The hepatic lesions were similar in appearance to those observed in the spleen; however, they were less numerous and somewhat smaller. The substance that composed these foci resembled caseation necrosis rather than pus. One very small abscess was observed in the cortex of the left kidney. Other organs appeared to be normal. The brain and spinal cord were not examined.

Microscopic Examination: Fresh tissue was fixed in Orth's fluid, embedded in paraffin and stained with hematoxylin and eosin. The lesions are essentially the same in all anatomical situations, as observed in sections of the lungs (Fig. 2), liver (Fig. 3), spleen and left kidney. They consist of focal, sharply circumscribed zones of exudation with usually advanced necrosis of the exudate centrally and granulomatous proliferative changes peripherally. Early lesions are composed almost entirely of polymorphonuclear neutrophilic leukocytes without necrosis or peripheral granulomatous reaction. The usual and typical lesion, however, consists of the three zones: (1) a central zone of necrosis composed of granular, eosin-staining acellular material in which chromatin dust-like fragments of cell nuclei persist; (2) a wide zone about the central zone of necrosis composed of polymorphonuclear neutrophilic leukocytes in which a few lymphocytes and large phagocytic mononuclear leukocytes are also found; and (3) a peripheral zone about each lesion consisting of gradually diminishing numbers of leukocytes and gradually increasing numbers of endothelioid cells, fibroblasts, and occasionally also young blood vessels. The latter area varies from lesion to lesion, so that in some it comprises only a small portion, while in others it represents a well defined zone. The three zones are not especially distinct from one another, but each appears to blend gradually with the adjacent one. No further encapsulation exists, but adjacent to each lesion there is atrophy of parenchymal cells together with retrogressive cellular changes. Around some of the pulmonary lesions, however, there is an organizing pneumonic process for a considerable distance. In the peripheral portions of the lesions large compact aggregations of bacteria in colony formation are numerous. These masses are composed of faintly stained, small Gram-negative bacillary forms with a tendency to bipolar staining. Single organisms in the tissue are difficult to find, but a few are seen as plump, Gram-negative rods. They are not acid-fast.

BACTERIOLOGICAL DATA

In a culture from the blood, made 24 hours before death, Gram-negative, rod-shaped forms appeared in the broth after 2 days. At the same time, a few colonies of similar organisms appeared in the agar plates. There was less than one colony for each cc. of blood used. At autopsy cultures were obtained from the blood, spleen, liver and lung. From all of these sources organisms were obtained having the following characteristics: Medium sized, Gram-negative, non-motile rods, occurring singly or in short chains in broth cultures. On blood agar, after 24 to 48 hours, the colonies were 1 to 2 mm. in diameter, round, convex and milky white. There was no effect on the blood. On nutrient agar the colonies were similar but smaller. In nutrient broth a pellicle and sediment were formed. Ehrlich's test for indol was positive after 3 days. On potato slants there was no visible growth after 6 days. Blood serum and gelatin were not liquefied after 15 days. On glycerin agar the growth was white, shiny, flat and membranous after 5 days. In sugar fermentations acid but no gas was produced in glucose, maltose, mannite and salicin. Neither acid nor gas was produced in lactose, saccharose, dulcitol and inulin. The cultural characteristics are summarized in Table I, in which the organism isolated from the present case is designated by the letter Z. For comparison the table includes the strain of *Actinobacillus* previously isolated from a human being, and in addition, typical strains of *Bacillus whitmori*, *Pfeifferella mallei* and *Actinobacillus* of bovine origin. Thompson⁷ has recently recommended that these organisms be included in the genus *Actinobacillus*.

The serological relationship of the organism was determined by immunizing rabbits to produce an agglutinating serum of high titer. Similar serums were prepared with a culture of *Actinobacillus* of bovine origin and a culture of *Pfeifferella mallei*. The results of cross agglutination tests are shown in Table II. Serum Z is the one prepared with the organism isolated from the present case. Table II also includes, for comparison, the results obtained with an organism from a previous case and with a culture of *Bacillus whitmori*. Attention is called to the fact that all of these serums give a positive complement fixation test with an antigen prepared from *Pfeifferella mallei*. Although it does not appear in the table, tests were made

TABLE I
Cultural Characteristics of Genus *Actinobacillus*

Culture	Motility	Glycerine agar	Gelatine	Blood serum	Potato	Indol	Sugar fermentation						
							Glucose	Maltose	Mannite	Lactose	Saccharose	Dulcitol	Inulin
I*	—	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	A**	A	A	A	A	o	o
99†	+	Wrinkled, yellow	Liquefied	Liquefied	Growth good, yellow	—	A	o	Sl. A	Sl. A	o	o	o
PD‡	—	Smooth, yellow	Not liquefied	Not liquefied	Growth good, yellow	Sl. +	o	o	o	o	o	o	o
M§	—	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	Sl. A	o	Sl. A	o	Sl. A	o	o
Z§	—	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	A	A	A	o	o	o	A

* I = *Actinobacillus* (bovine)

** A = Acid

† 99 = *Bacillus subtilis*

‡ PD = *Pfeifferella mallei*

§ M and Z = *Actinobacillus* (human strains)

which showed that serum Z did not agglutinate *Alcaligenes abortus* and *Pasteurella tularensis*.

To summarize the information contained in the tables it may be said that the organism under consideration shows a group relationship with *Pfeifferella mallei* and with *Bacillus whitmori*. The latter organism is the cause of a glanders-like disease in southeastern Asia called melioidosis. It also resembles more strongly *Actinobacillus*

TABLE II
Serological Reactions of Actinobacillus

Cultures	Rabbit serums prepared with culture					Complement fixation with mallei antigen
	I	99	PD	M	Z	
I*	10,240	0	20	40	160	44400
99†	0	2560	40	0	0	44444
PD‡	20	2560	2560	20	20	44444
M§	0	80	0	320		44400
Z§	80		80		5120	44400

* I = *Actinobacillus* (bovine)

† 99 = *Bacillus whitmori*

‡ PD = *Pfeifferella mallei*

§ M and Z = *Actinobacillus* (human strains)

lignièresii, the cause of bovine actinobacillosis, but differs from the latter in certain sugar fermentations. From the study of two strains obtained from human beings it seems most logical to consider these organisms as *Actinobacillus lignièresii*, which show slight cultural and antigenic differences from the strains of bovine origin.

It is possible that infections due to *Actinobacillus lignièresii* have not been recognized in the past, owing to the superficial similarity of this organism to those of the coli group. All Gram-negative, non-motile, aerobic organisms that produce only acid in sugar mediums should be investigated more fully as to cultural and serological reactions.

EXPERIMENTAL DATA

Triturated preparations obtained from the spleen, liver and lung of the patient at autopsy, ante mortem blood, and pure cultures as recovered from the ante mortem blood culture and from the lesions at autopsy, were proved to be pathogenic for rabbits, guinea pigs and mice. In these animals granulomatous abscesses and tubercle-like granulomas were evolved. The granulomatous features in the lesions were frequently much more prominent than they were in the lesions of human beings; nevertheless, they were similar if not identical with one another. A clearly evident Strauss reaction was never elicited, although in two guinea pigs mild periorchitis was revealed at autopsy. Bacterial masses in ball-like colony formation were found in many of the infectious foci of the experimental animals, similar in appearance to those that were seen in the lesions of human beings. Well defined peripheral clubs were not associated with these colony formations, as in bovine actinobacillosis, although suggestions of early club formations were sometimes observed. In cover-slip preparations of the exudates the organisms appeared usually as single, plump, faintly stained Gram-negative rods, sometimes in pairs or in short filaments, many revealing bipolar staining characteristics.

The lesions in guinea pigs and rabbits also, in a general way, resembled those described for experimental glanders. The experimental disease produced by the organism from our case was chiefly distinguished from experimental glanders by failure to produce a characteristic Strauss reaction in guinea pigs, the colonization of the bacteria in aggregations in the lesions and by revealing greater pathogenicity for rabbits than for guinea pigs.

The strain of *Actinobacillus* from our case was of greater pathogenicity than generally has been described for the *Actinobacillus* of bovine origin. Lignières and Spitz ^{8,9} found that their cultures had variable degrees of virulence for bovines as well as for small laboratory animals, although they distinctly described the evolution of fatal peritonitis on intraperitoneal inoculation of guinea pigs, with death of the pigs in from 12 to 24 hours when large doses were used, and in from 5 to 7 days when smaller doses were used. The character of the peritonitis produced in their animals was apparently comparable with that revealed by ours. The suggestion of a mild Strauss reaction in two of our animals is also in accord with their description

of periorchitis, which developed in guinea pigs inoculated intraperitoneally, although this reaction in their animals was of much greater severity. In the lesions of guinea pigs inoculated intraperitoneally Lignières and Spitz found the tufts or aggregations of microorganisms with peripheral clubs similar, although less distinct, to those evolved in bovine lesions. In our experimental guinea pigs, especially those inoculated intraperitoneally, the aggregations of bacteria in compact colony formation were very prominent in many of the lesions, although the zone of peripheral clubs was either only faintly suggestive or not revealed. Neither Magnusson¹⁰ nor Griffith¹¹ was able to confirm this observation of Lignières and Spitz. The formation of subcutaneous abscesses in guinea pigs, which developed on subcutaneous introduction of the organisms such as we describe, was also produced by bovine strains in the experiments of other investigators.

Rabbits inoculated with the strain from our case were more readily infected than other observers have found them to be when working with bovine strains of the organism. There is perhaps no record of the production of lesions by the *Actinobacillus* in rabbits comparable to those exhibited by our animals. It is therefore apparent that in this instance we have a strain of virulence of high degree, which may account in part for the fulminating infection of our patient and also for the ease with which lesions were evolved in animals. In the case of *Actinobacillus* bacteremia studied by Thompson and Willius the organism was much less virulent for both man and laboratory animals. This is what one would usually expect, judging from the experience of other observers in working with bovine strains.

Rabbits: These animals appeared to be most susceptible to the infection (Table III). In Rabbit 1, which received an intravenous inoculation of 1 cc. of a 24 hour pure brain broth culture, the greatest virulence of the organism was manifest, death of the animal resulting in 29 hours. At autopsy small, scarcely visible, granulomatous foci were found in the liver, lungs and spleen. Microscopically these consist of focal areas of necrosis with early polymorphonuclear leukocytic exudation and beginning proliferation of endothelioid cells. The lesions are discrete, multiple and particularly prominent in the liver and spleen. Rabbits 2, 3 and 4, which received 0.3, 0.8 and 1.5 cc. of a pure 24 hour brain broth culture intravenously, lived 15, 11 and 5 days respectively. Clinically, all of these rabbits revealed

a slowly developing but progressive general reaction in which loss of appetite, emaciation and listlessness were the chief symptoms. At autopsy lesions were found similar to those seen in Rabbit 1, but more advanced. In each of the animals lesions were observed in the liver, lungs and spleen. In Rabbits 2 and 3 the kidneys also were involved, and in Rabbit 2 the cecum. In Rabbits 2 and 3 the lesions were particularly well defined. Grossly in each instance the spleens were enlarged to approximately five times normal size and were mottled by grayish areas of necrosis, sometimes discrete, but due to the enormous number of lesions becoming confluent (Fig. 4). In the livers and lungs the lesions were extremely small and appeared as a fine grayish white mottling. In the kidneys and cecum larger grayish necrotic foci appeared. Microscopically there is considerable variation in the appearance of the lesions, but they are still compatible with stages of the same granulomatous process. Briefly stated, the lesions consist of a central zone of necrosis in which nuclear detritus is rather prominent. Surrounding this polymorphonuclear leukocytes have collected and peripherally a rather well defined zone of endothelioid cells is observed. In some instances the outer portion of this area is composed mostly of fibroblasts. Interspersed with the endothelioid cells and fibroblasts are a few polymorphonuclear leukocytes, lymphocytes, mononuclear leukocytes and plasma cells. The zone of necrosis varies so that in some instances it occupies almost completely the entire focus with just a narrow rim of cellular elements, whereas in other lesions it is extremely small or almost absent. When the latter prevails the center of the lesion is either occupied by polymorphonuclear leukocytes as the predominating cell, or the entire structure is evolved as a tubercle, consisting almost entirely of endothelioid cells. Occasionally Langhans' giant cells are observed in the peripheral granulomatous zone. Bacterial colonies are rarely identified with the lesions in rabbits, but when found, as in Rabbit 4, they appear to occupy the central zone and are surrounded by an area of necrosis in which a few polymorphonuclear leukocytes are visible. The bacterial colonies are composed entirely of small, Gram-negative bacillary elements, without peripheral clubs.

It is easily possible to fulfil Koch's postulates by the rabbit inoculations. Pure cultures, as obtained from the lesions of the patient, caused the death of Rabbits 1 and 2 and produced in them lesions

TABLE III
Inoculation of Animals

Animal	Date	Material	Culture	Dosage	Method of inoculation	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
Rabbit 1	1/14/33	Liver, patient	24 hour pure brain broth	cc. 1.0	Intra-venous	Severe general reaction	Died 1/15/33	1	Granulomatous foci liver, lungs, spleen	Liver and spleen
Rabbit 2	1/15/33	Liver, patient	48 hour pure brain broth	0.3	Intra-venous	Slow general reaction	Died 1/30/33	15	Granulomatous foci liver, cecum, lungs, spleen, kidney	Spleen
Rabbit 3	1/20/33	Spleen, Rabbit 1	24 hour pure brain broth	0.8	Intra-venous	Slow general reaction	Died 1/31/33	11	Granulomatous foci liver, lungs, spleen, kidney	Liver
Rabbit 4	1/25/33	Spleen, Rabbit 1	24 hour pure brain broth	1.5	Intra-venous	Slow general reaction	Died 1/30/33	5	Granulomatous foci liver, lungs, spleen	Blood, liver, spleen
Rabbit 5	2/11/33	Spleen, patient	24 hour pure brain broth	2.5	Subcutaneous	Abscess site inoculation, no general reaction	Killed 3/14/33	31	Granulomatous foci lung, subcutaneous abscess	Subcutaneous abscess
Rabbit 6	2/11/33	Spleen, patient	24 hour pure brain broth	2.5	Subcutaneous	Abscess site inoculation, general reaction, rapid terminal failure	Died 2/18/33	7	Granulomatous foci liver, spleen, abscess site inoculation	Blood, liver, spleen, subcutaneous abscess
Mouse 1	1/12/33	Blood patient, ante mortem	Original 24 hour brain broth	0.2	Subcutaneous	Slight local induration, no general reaction	Killed 2/9/33	28	Granulomatous foci spleen	Accidentally lost
Calf 1	2/1/33	Spleen, patient	24 hour pure brain broth	5.0	Subcutaneous	Mild general reaction, fever 2 days, bloody diarrhea, local swelling, re-covered	Killed 3/29/33	57	No lesions	Negative

similar to those revealed at autopsy of the patient. Pure cultures of the organism as inoculated were obtained from the liver and spleen. Rabbits 3 and 4 were inoculated with the culture that had been isolated from Rabbit 1, and there also the lesions were unmistakably almost identical with those observed at autopsy of the patient. The organism was again isolated from the lesions of these rabbits in pure culture and proved to be identical with the original culture. In only one of the intravenously inoculated rabbits, Rabbit 4, was the blood culture positive at the time of death.

Subcutaneous inoculation of two rabbits proved fatal in one, the rabbit dying on the 7th day of septicemia. A local abscess was produced, as well as lesions in the liver and spleen. The other rabbit that received subcutaneous inoculation survived and was killed on the 31st day after inoculation. It revealed a well defined encapsulated local abscess and a few healing granulomatous abscesses in the lungs. In the lesions of Rabbit 6 colonization of the organisms in the disseminate lesions was a prominent feature. In Rabbit 5 organisms were not identified in the tissue sections. In both, however, cultures were positive.

Mice: Judging from the inoculations of one white mouse (Table III) mice are practically non-susceptible. Subcutaneous inoculation did not appear to provoke any illness or local lesion. The animal was killed on the 28th day; a few small, typical, chronic granulomatous foci were found in the spleen only.

Cattle: One calf was inoculated and results were essentially negative (Table III).

Guinea Pigs: These animals exhibited a variable susceptibility, although in all but one some lesions were produced. The results of subcutaneous inoculations are summarized in Table IV. Although local lesions were produced in all but Guinea pig 2 it will be noted that none of the animals died from the infection. Guinea pig 8 died on the 16th day after inoculation; pneumonia and not *Actinobacillus* infection was the cause of death. The pigs usually manifested slight disability, chiefly refusing food and losing weight for a few days, then generally recovered. The most severe reactions were encountered in Guinea pigs 1 and 4. Both were very ill when they were killed and probably would have died as a result of actinobacillosis. The severity of the illness of Guinea pig 1 was accounted for at autopsy by general peritonitis.

TABLE IV

Subcutaneous Inoculation of Guinea Pigs

Guinea pig	Date	Material	Material inoculated	Dosage	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
1	1/12/33	Spleen, patient	Triturated abscess spleen	Saline suspension 1 cc.	Abscess site inoculation, severe general reaction	Killed 1/18/33	6	Granulomatous foci liver, spleen, lungs, subcutaneous abscess, general peritonitis	Subcutaneous abscess, peritoneum, liver, spleen, blood
2	1/12/33	Lung, patient	Triturated abscess lung	Saline suspension 1 cc.	Small abscess site inoculation, mild general reaction, recovery	Killed 3/14/33	61	No lesions	Negative
3	1/12/33	Blood, patient, ante mortem	Original 24 hr. brain broth ante mortem culture	Brain broth culture 1 cc.	Small abscess site inoculation, drainage 50th day, no general reaction	Killed 3/14/33	61	Granulomatous foci liver, lungs, subcutaneous abscess	Subcutaneous abscess
4	1/12/33	Liver, patient	Triturated abscess liver	Saline suspension 1 cc.	Large abscess site inoculation, severe general reaction	Killed 2/4/33	23	Granulomatous foci liver, spleen, lung, subcutaneous abscess	Negative (contamination)
5	1/25/33	Blood, patient, ante mortem	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess and ulcer site inoculation, mild general reaction	Killed 2/9/33	15	Granulomatous foci liver, spleen, lungs, cutaneous ulcer	Negative (cultures lost)
6	1/25/33	Liver, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess and ulcer site inoculation, mild general reaction	Killed 2/20/33	26	Granulomatous foci liver, lung, cutaneous ulcer	Negative
7	1/25/33	Spleen, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess site inoculation, mild general reaction	Killed 2/20/33	26	Granulomatous foci liver, cutaneous abscess	Subcutaneous abscess
8	1/25/33	Lung, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess site inoculation, severe general reaction	Died 2/10/33	16	Granulomatous foci liver, lymph node, spleen, cutaneous abscess, pneumonia	Subcutaneous abscess

Subcutaneous abscesses formed at the sites of inoculation in all of the animals except Guinea pig 2, in which lesions could not be found at autopsy. In two instances the abscesses began to drain after a few days and a large purulent ulcer with necrotic base and undermined borders formed. In Guinea pig 6 the ulcer began to heal about the 20th day and when the animal was killed, on the 26th day, the ulcer was almost healed. In Guinea pig 5, however, the ulcer remained about 2 cm. in diameter and appeared to be progressing at the time the animal was killed. In Guinea pig 3 a small abscess developed which persisted, and on the 50th day a small sinus appeared, through which purulent material drained. In the eight guinea pigs inoculated subcutaneously small, discrete, grayish white, necrotic foci were found in the livers of seven, in the lungs of five, and in the spleens of four. One of the guinea pigs had general peritonitis of serofibrinopurulent type and one had an enlarged inguinal lymph node containing necrotic foci.

Microscopically the lesions in these animals are similar to those in the rabbits. The local subcutaneous abscesses are composed centrally of a large area of necrotic exudate in which a few polymorphonuclear leukocytes are preserved, but usually only nuclear detritus and eosin-staining granular material are present. Surrounding this is a wide zone of well preserved polymorphonuclear leukocytes that blends peripherally with a zone of granulation tissue in which fibroblasts and young blood vessels form the supportive structure. Intermingled with these cells are polymorphonuclear leukocytes, large mononuclear phagocytic cells, plasma cells, lymphocytes and other cells, sometimes quite numerous, interpreted as endothelioid. In the outer portions bacterial aggregations exist in colony formation; these are Gram-negative and not associated with peripheral clubs. In the viscera the lesions tend toward chronic abscesses, with central collections of polymorphonuclear leukocytes and peripheral granulomatous reaction. In some of the lesions necrosis is a prominent feature, but in others it is slight. In the older lesions the peripheral zone has become distinctly fibrous, and in others this fibrosis has progressed to involve most of the focus, as though to form a cicatrix of a healed lesion. However, in the more severe general infections, as in Guinea pigs 1 and 4, all stages of lesions exist from focal areas of necrosis to abscesses, as described. Intermediate lesions frequently resemble endothelioid tubercles, or wide zones of

TABLE V

Intraperitoneal Inoculation of Guinea Pigs

Guinea pig	Date	Culture	Culture dosage 1 cc.	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
1	1/14/33	Spleen, patient	24 hour pure brain broth	Severe general reaction, con- junctivitis	Killed 1/18/33	4	Fibrinopurulent peritonitis, granu- lomatous foci in peritoneum, liver, spleen, lungs, lymph nodes	Liver, peri- toneum, spleen
2	1/14/33	Spleen, patient	24 hour pure brain broth	Severe general reaction, con- junctivitis	Died 1/20/33	6	Fibrinopurulent peritonitis, granu- lomatous foci in liver, spleen, lungs, kidneys, lymph nodes	Spleen
3	1/25/33	Blood, patient	24 hour pure brain broth	Severe general reaction	Died 2/3/33	9	Fibrinopurulent peritonitis, granu- lomatous foci in liver, spleen, lungs	Liver, perito- neum
4	1/25/33	Liver, patient	24 hour pure brain broth	Severe general reaction	Died 1/31/33	6	Fibrinopurulent peritonitis, granu- lomatous foci in liver, spleen, lungs, adrenals, lymph nodes	Blood, liver, perito- neum
5	1/25/33	Spleen, patient	24 hour pure brain broth	Severe general reaction	Died 1/30/33	5	Fibrinopurulent peritonitis, granu- lomatous foci in liver, spleen	Blood, liver, perito- neum
6	1/25/33	Lung, patient	24 hour pure brain broth	Mild general reaction, re- covery, subcutaneous ab- scess with draining sinus	Killed 2/20/33	26	Subcutaneous abscess with drain- ing sinus, granulomatous foci in liver, lungs, adrenals, local peri- tonitis, periorchitis	Negative

central necrosis with a slight exudative and granulomatous proliferative reaction peripherally. In most instances colonies of bacteria are associated with the lesions; in the early lesions in the center of the necrotic focus, and in older lesions toward the border of the zone of necrosis or in the area of exudation. These are frequently found as large compact masses not retaining the Gram stain, and sometimes with just a faint suggestion of peripheral clubs.

Following intraperitoneal inoculation of guinea pigs with pure cultures (Table V), the animals became ill in from 24 to 48 hours; they refused food, were weak and listless, the hair became roughened, they lost weight and the abdomen became tender. All but two of the animals, Guinea pigs 1 and 6, died in from 5 to 9 days. Guinea pig 1 was moribund at the time it was killed and would have died within a few hours. Guinea pig 6, however, after an initial slight general reaction, apparently recovered with only a local abscess and draining sinus at the site of inoculation. It was killed on the 26th day. In none was the Strauss reaction elicited clinically. At autopsy all the animals were found to have peritonitis. In only one, Guinea pig 6, was the reaction local; it was confined to an area beneath the parietal peritoneum of the ventral wall, and adherent loops of small intestine and omentum. A sinus tract through the peritoneum and abdominal muscles formed a subcutaneous abscess and a draining sinus through the skin. The peritonitis was of a peculiar type. The peritoneal cavity contained a few cc. of clear amber fluid. The surface of the liver and spleen and usually the omentum and stomach were covered by a thick, adherent, grayish yellow, fibrinopurulent exudate. These structures were frequently adherent to one another. Over the coils of the small intestines there were frequently isolated or discrete, small, grayish yellow foci that resembled tubercles. In two guinea pigs (Guinea pigs 1 and 6) the same appearance was presented on the tunica of the testis and epididymis. The livers of all the animals were involved by fine grayish yellow points of apparent necrosis. Similar lesions, although sometimes more distinct, were observed in the lungs and spleen of five animals. In two animals the suprarenal glands were slightly involved. In one animal the mediastinal lymph nodes were involved and in one, Guinea pig 6, the inguinal nodes. In two animals, as has been noted, periorchitis existed.

Microscopically, except in Guinea pig 6, the lesions are usually relatively acute, but throughout the various involved organs and the

various animals, lesions of varying age are presented. These are observed in their simplest form as areas of necrosis at the point of lodgment of bacterial masses in the capillaries, to focal abscesses or focal granulomatous, tubercle-like structures. The early lesions consist of a central compact aggregation of bacteria surrounded by a zone of necrosis in which a few polymorphonuclear leukocytes are seen. Slightly older lesions reveal a peripheral zone of endothelioid cells, with perhaps a greater number of polymorphonuclear leukocytes collected around the bacterial colony (Fig. 5). In other instances lesions of this stage reveal only central necrosis with a rim of polymorphonuclear leukocytes and endothelioid cells, or with the central zone composed entirely of polymorphonuclear leukocytes without necrosis. Central or eccentric masses of bacteria are sometimes observed in the latter lesions, although they not infrequently are entirely absent. Still older lesions are in certain instances predominately endothelioid tubercles, sometimes with central polymorphonuclear neutrophilic exudation and at other times with almost solid endothelioid cells. In Guinea pig 6 the lesions are more chronic (26 days). They consist of central polymorphonuclear leukocytes with considerable necrotic cellular débris, a peripheral rim of endothelioid cells and fibroblasts or fibrous connective tissue. These lesions are in the liver, suprarenal glands, lungs, tunica of the testes and subcutaneous tissue. In these chronic lesions the masses of bacteria are not visible.

THE PATHOLOGICAL CHANGES IN BOVINE ACTINOBACILLOSIS

The lesions of bovine actinobacillosis are grossly like those of actinomycosis, but although true bovine actinomycosis usually involves the jaw bones, where it produces rarefying osteitis, bovine actinobacillosis usually affects the soft tissues of the head and neck. Specifically the parts most often affected are the tongue, palate, pharynx, cheeks, skin of face and lymph nodes of the submaxillary and cervical regions. The lungs have been described also as the site of affection.

Grossly the smaller bovine lesions may be described as appearing like areas of soft granulation tissue with central suppuration, the central zone of exudation gradually enlarging and the peripheral zone of granulation tissue gradually developing a thick fibrous capsule. The pus is thick, viscid or gelatinous and greenish yellow. In it the sulphur granules are found.

The microscopic characteristics of the lesions, as determined in bovine material from which Thompson isolated the *Actinobacillus* in pure culture, will be appended for comparative study. These corresponded with the previously published descriptions of such lesions. They also were essentially the same as those that were found in our patient, and those that were produced in our experimental animals. The central portion of the lesion, which was essentially a granulomatous abscess, was composed of exudate in which fairly well preserved polymorphonuclear leukocytes predominated. Small granules, the colonies of bacteria with peripheral clubs, were fairly abundant in the exudate that adhered to the wall of the abscess. This wall consisted of granulation tissue of loose texture, the principal supportive structure of which consisted of fibroblasts and newly formed blood vessels, throughout which were interspersed lymphocytes, large phagocytic mononuclear leukocytes, plasma and endothelioid cells. Polymorphonuclear leukocytes gradually diminished in number as the peripheral portions were reached, except for focal aggregations. The uniform structure of the wall of the abscess was somewhat altered by the presence of small abscesses, similar, although much smaller, to the central large one. This gave the entire wall more or less of an alveolar appearance. The smaller abscesses were composed of a central portion in which one to several compact bacterial aggregations with peripheral clubs existed. When these were stained by Gram's method only Gram-negative bacillary forms in compact aggregations with peripheral clubs were revealed. They were entirely devoid of Gram-positive branched filaments. Surrounding the bacterial colonies there were well preserved polymorphonuclear leukocytes, some necrosis and occasionally giant cells. Peripherally there was an encircling zone of slightly more compact granulation tissue than was found elsewhere in the wall of the large abscess, with a tendency to strands of fibrous connective tissue as a final encapsulation for the alveolar structure. Surrounding the entire lesion was a dense encapsulation of fibrous tissue.

EPIDEMIOLOGY

The patient's immediate family was carefully questioned concerning possible sources of infection, but without eliciting information of value. It was stated most emphatically that he had had no contacts with cattle or other animals, that he had never drunk milk as a

beverage and that all milk used at his home was pasteurized dairy milk. He had always insisted on his meats being well cooked. There had been no local injuries or lesions about the hands or other exposed surfaces of the body.

The method of natural spread of the disease among cattle is not known, but it is presumed that the organism is strictly parasitic and contact with infected animals or carriers constitutes the mode of dissemination. The disease usually occurs sporadically in cattle and is not highly contagious, although Lignières and Spitz report its occurrence in cattle in epizootic form.

SUMMARY

Three cases of actinobacillosis in man are now well authenticated. The case reported here offers the first opportunity for pathological study of the lesion in a human being. The lesions are essentially similar to those that have been described in cattle, except that in man the lesions are much more widespread, and sulphur granules, such as occur in bovine lesions, apparently do not occur. In the present case the lesions may be described as granulomatous abscesses severely affecting the lungs, liver and spleen. Lesions similar to those observed in man and cattle were produced in experimental animals. Glanders and tularemia were considered in the differential diagnosis, but both seemed to be definitely ruled out by the bacteriological and serological investigations. The organism isolated from our case reveals a close cultural and antigenic relation to *Actinobacillus lignièresi*, and a more distant relation to *Pfeifferella mallei* and *Bacillus whitmori*. Thompson has previously shown that *Actinobacillus lignièresi* of bovine and human origin, *Pfeifferella mallei* and *Bacillus whitmori* are antigenetically interrelated, and has proposed that they be included in a common genus. This relationship, as far as the organism in our case is concerned, is also confirmed from the pathological viewpoint, for the lesions in man and in experimental animals were similar to glanders, as well as exhibiting similarities to bovine actinobacillosis. Although the organism under consideration revealed minor cultural differences and somewhat different antigenetic phenomena from a typical strain of *Actinobacillus lignièresi* of bovine origin, nevertheless, it seems justifiable to regard the organism in this case as a variant of the usual bovine strain of *Actinobacillus lignièresi*.

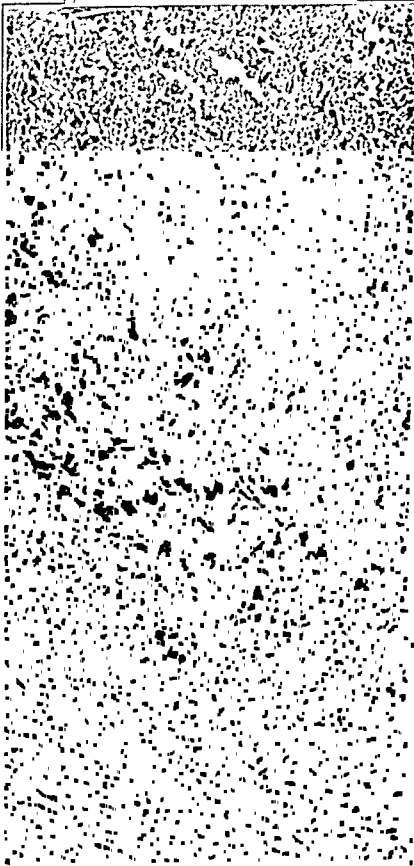
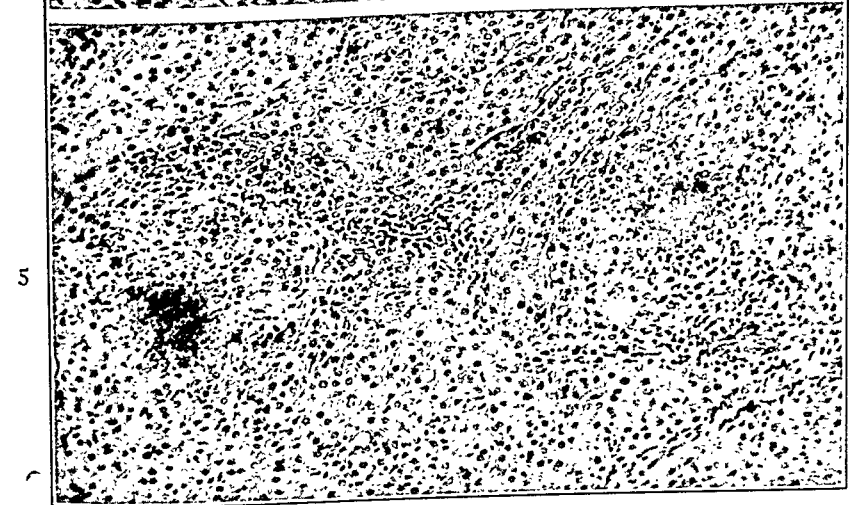
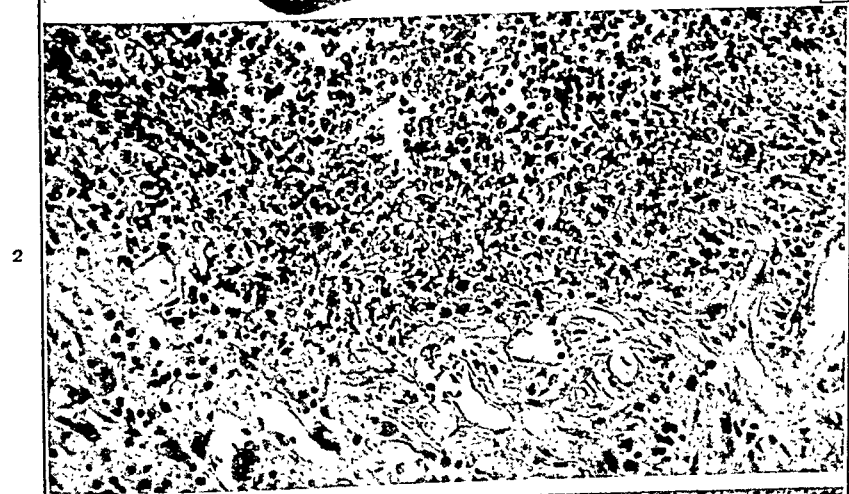
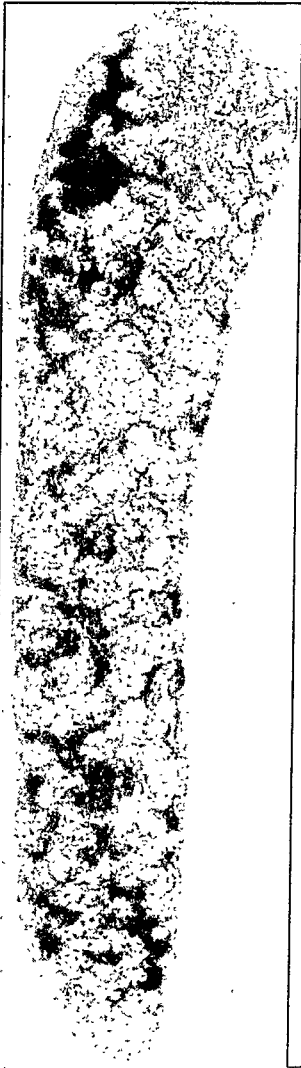
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DESCRIPTION OF PLATE

PLATE 101

- FIG. 1. Spleen of patient revealing multiple granulomatous abscesses.
- FIG. 2. Granulomatous abscess from lung of patient. In the center of the abscess polymorphonuclear neutrophilic leukocytes predominate with a few mononuclear phagocytes and lymphocytes. Peripherally endothelioid cells and fibroblasts are seen just within and intermingling with a granulation tissue encapsulation. Hematoxylin and eosin stain. $\times 160$.
- FIG. 3. Portion of large granulomatous abscess from liver of patient. Central necrosis, zone of leukocytes and early peripheral endothelioid cell proliferation may be seen. Large masses of bacteria in colony formation, more deeply stained, may also be seen. Hematoxylin and eosin stain. $\times 45$.
- FIG. 4. Spleen of Rabbit 3 inoculated intravenously with pure culture of *Actinobacillus*, as isolated from the liver of the patient after passage through Rabbit 1. The rabbit died the 11th day after inoculation. The resemblance to the lesions in the patient's spleen may be noted (Fig. 1).
- FIG. 5. Liver of guinea pig inoculated intraperitoneally. Two well defined lesions are revealed. Colonies of *Actinobacillus* may be noted in the centers of the lesions. Surrounding the bacterial colonies are polymorphonuclear neutrophilic leukocytes and necrosis, and at the periphery is a well defined zone of endothelioid cell proliferation. Hematoxylin and eosin stain. $\times 110$.



ERYTHROLEUCOBLASTOSIS IN THE NEWBORN *

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Numerous cases of congenital hydrops have been described since 1878, but Schridde ¹ in 1910 directed attention to the changes in the blood-forming tissue in congenital hydrops, accompanied by a high degree of anemia. Since then it has often been called Schridde's type of congenital hydrops, in contradistinction to general edema. His findings have been confirmed time and again by numerous investigators, who also have described a reparative growth of erythroblastic tissue and a general edema, which had no etiological relation to congenital syphilis or to a coexistent maternal nephritis.

After careful investigation of a case Rautmann ² in 1912 expressed the opinion that the real condition was a primary erythroblastic process and therefore coined the term "erythroblastosis."

Thirty-four cases from the German literature were reviewed by Hartmann ³ in 1928. He noted the frequent coincidence of maternal renal disturbances, but later concluded that the fetal condition was primary and the maternal renal disease secondary, due to a toxicosis produced by the fetus. Oberndorfer ⁴ found congenital hydrops in one of twins and concluded, therefore, that a maternal etiological factor was out of the question.

In 1921 von Gierke ⁵ found the pathological changes described by Schridde in a non-hydropic baby with "Kernikterus," and he established the hypothesis that these diseases might be related and that they might represent different complexes of symptoms, but be due to the same cause. The observation of the coincidence of icterus gravis neonatorum, which can occur with or without "Kernikterus," with fetal erythroblastosis has been so abundantly confirmed that the occurrence is undoubted. He believed the condition not to be a primary destructive anemia.

Kleinschmidt ⁶ found a high degree of erythroblastosis in icterus neonatorum, the blood picture corresponding to that of an embryo between three and five months. Volhard ⁷ believed there existed a

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similarity between cases of congenital edema and icterus gravis neonatorum and that there was a pronounced familial disposition, as frequently several offspring of one mother showed one or the other condition.

Von Gierke in 1930⁸ examined a newborn girl baby who died one hour after birth. This child was a sister of the child with "Kernikterus" described by him in 1921. The liver and spleen were greatly enlarged, with a tear in the capsule from which there was some bleeding. Blood smears showed numerous normoblasts, megaloblasts and a large number of myelocytes and myeloblasts. The spleen, liver and bone marrow showed a high degree of erythroblastic and myeloblastic growth, with smaller masses of these cells in the kidney, skin and heart muscle. This form von Gierke called leucoblastosis, having a relation on the one hand to erythroblastosis and on the other to a few cases of fetal leukemia, which had been described. He concluded that there is a disturbance of fetal hematopoiesis in which either the erythroblastic or the leucoblastic apparatus, or both, are concerned. Accordingly he divided fetal blood diseases into:

1. Fetal erythroblastosis
2. Fetal erythroleucoblastosis
3. Fetal leucoblastosis

The first and second were frequently accompanied by congenital hydrops or by icterus gravis neonatorum. The third was like extrauterine leukemia. The etiology of this condition was not known.

Furthermore, von Gierke concluded that there was a tendency for several cases to occur in the same family, or cases of both congenital hydrops and icterus neonatorum. In the majority of cases the mother was perfectly well so that maternal illness was only an incidental occurrence. He believed that these conditions were each a part of one disease entity, which under changes in circumstances involved different components of the hematopoietic system. Microscopically these conditions presented a marked increase in immature forms of the erythroblastic and leucoblastic series in the blood and also in the organs, particularly in the liver and spleen. The erythroblastic disease was frequently found with an accompanying congenital edema or an icterus gravis neonatorum. Also, in congenital hydrops there was frequently a slight icteric tint in the tissues. It seemed possible that both manifestations were due to the same fetal

blood disease; congenital hydrops appeared early, was intrauterine, causing a stillbirth or an infant living only a few hours, while icterus gravis neonatorum usually appeared in the first few hours after birth. With congenital hydrops and icterus gravis neonatorum there was frequently a hyperplastic placenta, but the reason for this was not known.

Von Gierke also reported a case of fetal erythroleucoblastosis in which the liver and spleen showed a marked increase in erythroblastic and leucoblastic myelogenous tissue in the hematopoietic areas. The kidneys, heart muscle, skin and gastric mucosa also showed extravascular myeloid nodules. A large part of the leucoblasts gave the oxydase reaction. The picture closely resembled that of myelogenous leukemia, except for the marked increase in erythroblasts in the blood and in the cellular infiltrations in the tissues.

In 1930 Hueper and Mullen⁹ described two cases of erythroblastosis with congenital hydrops and believed these findings were the effects of the toxic action of an unknown substance in the circulation upon the blood and the vascular walls, resulting in the persistence of the hematopoietic system at the high developmental level of the seventh month, or even at a higher level, as an attempted reparative or compensatory hyperplasia of this tissue. Iron pigment was found in the liver and spleen, and bile casts in the bile ducts of the liver, indicating the existence of a marked degree of blood destruction.

Six cases of erythroblastosis were reported by Ferguson¹⁰ in 1931, three of them showing jaundice, two with a generalized edema and one with neither jaundice nor edema. All six showed marked enlargement of the liver and spleen, and active hematopoiesis in bone marrow, liver, spleen and other organs. In two of his cases there was bile stasis in the liver cells and bile capillaries.

In 312 autopsies on the newborn Salomonsen¹¹ in 1931 found five cases of congenital hydrops with fetal erythroleucoblastosis. There was marked hemosiderosis in the liver but none in the spleen. One of his cases gave opportunity for examination of blood smears during life. The hemoglobin reading was 81 per cent and the red blood cell count was 3,765,000 with 123,700 nucleated cells, of which 49 per cent were nucleated red blood cells. All forms of immature blood cells were present. The livers and spleens of fifty-two normal newborn infants were examined histologically for iron pigment, but the

hemosiderin found in congenital hydrops did not differ enough from that occurring normally to draw any conclusions in regard to a primary destructive anemia from this one finding. Salomonsen believed that a familial relation existed between congenital hydrops and congenital icterus gravis. He concluded that it was correct to ascribe fetal erythroleucoblastosis to a primary constitutional disturbance of the "Anlage" of the hematopoietic system, being a special fetal disease group; and that this group included congenital hydrops and icterus gravis congenitas as well.

As evidence for the familial occurrence of this disease complex Plaut and Bullard ¹² in 1926 reported a family in which two infants died of icterus gravis and another had typical congenital hydrops with erythroblastosis. They believed that autopsy examinations of babies with icterus gravis familiaris and of non-hydropic babies of mothers who had previously given birth to hydropic infants, would reveal more instances of "Kernikterus" and give more evidence of a connection between fetal hydrops, erythroblastosis, icterus gravis familiaris, "Kernikterus" and other forms of jaundice in the first days and weeks of life. The origin of this disease complex was not known but the child affected was usually immature, the thymus small and there was no maternal, paternal or other condition to give any clue.

Clifford and Hertig ¹³ in 1932 suggested that a subsequent pregnancy might produce a different clinical type of disease. It seemed likely that it might result as a defect of the germ plasm or of the fertilized ovum. Erythroblastosis appeared to run the major portion of its course *in utero* and in the first days of life. It occurred in either sex and in any race, but the first born was usually spared. Congenital hydrops appeared relatively frequently, 1 to 1200 cases at the Boston Lying-In Hospital. Icterus gravis was considered relatively infrequent while, in the same hospital, there occurred seven proved cases of erythroblastosis during a period of nine months, or 1 in 340 births. These observers found the placental villi enlarged, edematous and somewhat hyperplastic, and also noted that some of these babies were covered with a thick, golden yellow, vernix caseosa, although this occurred also on a few apparently normal babies.

REPORT OF CASE

Clinical History: C., a baby girl, was born in the University Hospital at 10:21 P.M., Jan. 7, 1931. The mother, aged 17 years, had always been in good health. Her father, aged 53 years, had diabetes mellitus and her mother, aged 48 years, was well. She had one brother and one sister living and well. There was no history of any constitutional disease in the family. Nothing was learned regarding the baby's paternal ancestry as the mother was unmarried.

The mother's health had been good throughout pregnancy. The cervix was fully dilated 11 hours after the onset of labor. The head was born easily, but the body of the child was delivered with considerable difficulty because of a markedly distended abdomen. Following the birth of the child 2000 cc. of clear, straw-colored amniotic fluid were discharged from the uterus. The onset of respiration was delayed and it was necessary to resort to artificial respiration.

Examination of the child, following delivery, revealed a female infant weighing 2040 gm., total length 41 cm., vertex to rump 25 cm., biparietal diameter 7.5 cm., suboccipital-bregmatic 8 cm., occipital-mental 11 cm., and occipital-frontal diameter 10 cm. The circumferences were as follows: suboccipital-bregmatic 27 cm., occipital-frontal 29 cm., and bis-acromial 25 cm. The abdomen was markedly distended by fluid, was soft, the percussion note was dull and a definite fluid wave could be obtained. The greatest circumference of the abdomen was 35 cm.

The child had several attacks of cyanosis shortly after birth and it was necessary to resort to oxygen with 5 per cent carbon dioxide to stimulate respirations. At 4:45 A.M., Jan. 8, 1931, about 6 hours after birth, respirations ceased. Permission for autopsy was granted and this was performed at 11:00 A.M.

Laboratory Findings: Routine diagnostic blood Kahn test on the mother gave negative results, as did the Kahn test on blood from the umbilical cord. The latter specimen contained bile.

AUTOPSY REPORT

The external examination of the body showed nothing additional to the findings given in the clinical history. There was marked cyanosis of the head, face and neck, and also numerous brownish red spots in the skin over the abdomen. The scalp showed marked edema over the left parieto-occipital region, with areas of ecchymosis. The very large liver could be palpated through the thin abdominal wall.

Around the brain stem and over the inferior portion of the cerebellum there was a massive and relatively recent blood clot that entirely filled the available space beneath the tentorium and was subarachnoid in distribution. This hemorrhage apparently arose from numerous small pial veins. The cerebrum showed marked congestion and edema. The cerebellum showed diffuse subarachnoid hemorrhage. There was marked congestion and edema of the cerebellar substance.

The panniculus was scant and was of a very faint yellowish color, somewhat suggestive of icterus.

The peritoneal cavity contained 500 cc. of clear, golden yellow fluid without fibrin masses.

The cardiac apex was at the sixth intercostal space in the anterior axillary line. The heart was 50 per cent larger than the cadaver's right fist and lay nearly horizontally. The anterior aspect was composed of the right ventricle, which showed extreme hypertrophy and measured 7 mm. in thickness. The apex was composed entirely of the right ventricle. The left ventricular wall was 5 mm. thick. The muscle was pale red and showed no gross lesions. The mitral valve was normal in size. The flaps were negative. The aortic valve was normal in size. There was no stenosis of the first portion of the aorta and the cusps were normal. The tricuspid valve showed very marked dilatation, admitting the tip of the first finger. It appeared incompetent. The pulmonary valve showed moderate dilatation but the cusps showed normal development. The pulmonary artery was dilated to approximately twice the usual size. The ductus arteriosus was larger than the arch of the aorta and was approximately three times the size of the normal ductus. There was a redundant semilunar fold guarding the foramen ovale.

The spleen was approximately twice the normal size. The capsule was somewhat thickened. The parenchyma was dark red and no lymphoid structure could be seen. The stroma appeared to be relatively increased.

The liver was moderately enlarged, dark red in color and the capsule was smooth. On section minute pale areas 0.5 mm. in diameter were observed, but the exact location of these pale areas with reference to the lobule could not be determined. The ductus venosus was dissected and found to be patent. The portal vein was investigated outside of the liver and no thrombosis found.

The kidneys appeared normal in size and showed well marked congestion. The ureters were moderately dilated, the right more than the left.

MICROSCOPIC EXAMINATION

Histological sections were prepared with hemalum and eosin; erythrosin and hemalum; erythrosin, picric acid and hemalum; Weigert's elastica stains; and silver stains by the coverslip method.

Spinal Cord: Marked congestion of meninges and cord substance is present.

Brain and Meninges: There is marked congestion and edema of meninges and congestion of brain substance. Small petechial hemorrhages are found beneath the ependyma of the ventricles. There are increased wandering cells in the meninges. Small calcified thrombi occur in the brain substance. Extensive intrameningeal hemorrhage is present.

Heart: Shows congestion. The muscle appears well developed for the age of the subject.

Aorta: Negative.

Lungs: The lungs show acute passive congestion. Aspirated meconium and epithelial cells from the amniotic fluid are found. The peribronchial nodes show lymphoid hyperplasia. Small hemorrhages are present. The fat stain shows no fat emboli.

Tongue: Negative.

Pharynx: The lymphoid tissue is hyperplastic. There are multiple perivascular foci of active hematopoiesis.

Larynx: Acute passive congestion is present.

Esophagus: Hematopoietic foci are seen in the esophageal wall.

Thyroid: The colloid is more abundant than usual at birth.

Thymus: The medulla is hypoplastic with very small corpuscles of Hassall. Areas of active hematopoiesis occur around blood vessels in the medulla. Congestion is present.

Spleen: An intense exacerbation of chronic passive congestion is seen. The stroma is increased. There are small foci, chiefly perivascular, of active hematopoiesis. Special stains show no hemosiderin.

Diaphragm: The muscle appears well developed. There are small perivascular collections of hematopoietic tissue.

Umbilical Cord Stump: Fresh thrombi without organization are seen.

Appendix: Marked mucin formation and passive congestion is present.

Small Intestine: There are small areas of hematopoiesis in the subserosa. There are lymphoid hyperplasia, marked passive congestion and also edema of the subserosa.

Duodenum: Marked chronic passive congestion is present.

Stomach: The stomach shows marked passive congestion and increased mucin formation.

Pancreas: Diffuse hematopoiesis is present throughout the pancreas. There is congestion and some increase in young connective tissue. The portal vein radicles are greatly distended and their walls thickened. The arteries show hypertrophy of the walls with marked development of the internal elastic lamina.

Liver: The prominence of the islands of Glisson is found to be due to infiltration with lymphocytes and cells of types found in bone marrow, an active and extensive hematopoiesis. There are also many myeloid giant cells present and many nucleated forms with eosinophilic cytoplasm. In the peripheral portion of the lobule many liver cells contain a yellow-brown pigment. By special staining it is found that a large part of this pigment is hemosiderin, the remainder being largely formol-precipitated hemoglobin. Small collections of bile pigment are present but there is no evidence of an obstructive jaundice. In some of the islands of Glisson the epithelium of the bile ducts shows degenerative changes and has been in part replaced by large spherical cells with large, deeply staining nuclei. These cells vary slightly in size, and in some the cytoplasm appears finely granular. Fat stains show a lipoidosis in the reticulo-endothelial cells.

Adrenals: Marked hypoplasia of the medulla and congestion of the cortex is seen. In the medulla are small vessels showing complete calcification of old thrombi.

Kidneys: Marked congestion is present and there is perivascular hematopoiesis, even in the cortex. There are large cells in the renal tubules, apparently the result of attempted repair of the damaged renal epithelium. These cells resemble so closely the large cells found in the bile ducts that it appears evident they must have had a similar origin.

Bone Marrow: The bone marrow is markedly cellular with active hematopoiesis throughout, the prevailing cells being of the red blood cell series.

Urinary Bladder: Negative.

Vagina and Rectovaginal Septum: There is hematopoietic tissue around most of the smaller blood vessels in the submucosa and fascia.

Internal Genitalia: Negative, except for congestion.

Lymph Nodes: The lymph nodes are hyperplastic, with a diffuse distribution of hematopoietic tissue throughout. The follicles are reduced in size and are represented by small aggregates of lympho-

blasts and lymphocytes, suggesting splenic corpuscles in their relation to arterioles.

Placenta: The villi of the placenta are somewhat plumper than normal. This is due more to edema than to increased stroma. Particularly in the larger stems, however, there is an increased number of cells that tend to be arranged in small aggregates about blood vessels. In these foci numerous cells with eosinophilic cytoplasm and numerous cells of the myelocyte series occur. There are no evidences of a syphilitic chorionitis and staining for spirochetes gives negative results.

Blood: (No clinical blood studies were made.) The blood as seen in the blood vessels of the tissues shows a very high proportion of nucleated cells in the red blood cell series.

Pathological Diagnoses: Fetal erythroleucoblastosis. Marked hematopoiesis in liver, spleen, kidney, bone marrow, lymph nodes, thymus, diaphragm, pharynx, small intestine, pancreas and placenta. Extreme portal passive congestion, stasis and ascites. Redundant plica semilunaris obstructing foramen ovale. Right ventricular hypertrophy. Dilatation of truncus pulmonalis and ductus arteriosus. Hyperplasia of elastica in abdominal arteries. Hypoplasia of adrenal medulla. Meningeal and subtentorial hemorrhages and subperitoneal petechiae. Marked acute passive congestion and edema of meninges and brain. Congestion of all organs. Compression cyanosis of the upper part of the body.

DISCUSSION

The cardiac changes are of considerable interest, the marked hypertrophy of the right ventricle, the redundant semilunar fold guarding the foramen ovale, and the dilatation of the pulmonary artery and the ductus arteriosus, but it must be borne in mind that these changes are probably the result and not the cause of the hematopoietic disturbance. If one were to assume that the redundant semilunar fold were primarily obstructive, dilatation of the pulmonary artery and of the ductus arteriosus would follow in sequence. On the other hand, all of these changes are subject to interpretation as compensatory manifestations. If subsequently this is found to be the case, these cardiac changes become analogous to those found in adult polycythemia. However, it is probable that the circulatory changes are causal in relation to the edema.

The very large spherical and polyhedral cells found in the small bile ducts and in the renal tubules require further discussion. Von Gierke¹⁴ found large cells in the liver, but from his description it is evident that he was referring to hypertrophic liver cells and not to bile duct epithelium. Lahs¹⁵ described infiltrations of "eosinophiles and giant cells" in the liver, but was referring to bone marrow giant cells. In one case Lommel¹⁶ noted a degeneration of renal epithelium but did not find large cells. As far as can be determined, such cells have not been described previously in connection with this group of diseases.

In the liver these large cells are found in the smaller bile ducts in the midst of the hematopoietic tissue of the islands of Glisson. Many such ducts appear to be practically obliterated by them. While there is much variability in size, the average diameter is about 50 microns. Where space permits each cell becomes roughly spherical, but where they are crowded together they assume an irregularly polyhedral form. The abundant cytoplasm is slightly basophilic with ordinary stains and contains numerous fat droplets that can be demonstrated by Sudan III. The nucleus of each cell is spherical and shows a large central chromatin mass without internal structure about which there is a broad and apparently hydropic nuclear membrane. In addition to the large basophilic nuclear mass, which has a diameter about one-third as great as that of the entire cell, one or more smaller basophilic granules are to be found just beneath the nuclear membrane or within its substance.

In the kidney the large cells occur in convoluted tubules at variable depths in the cortex. The tubules containing these large cells are themselves from three to six times wider than the neighboring tubules. In them the cells in question are found attached to the wall or free in the lumen. In many instances there is an almost continuous layer of intact epithelium below the large cells. As in the liver, these cells range around 50 microns in diameter and are spherical or irregularly polyhedral in shape. In all respects the nuclear and cytoplasmic characteristics are the same as for the cells occurring in the liver.

In both the liver and kidney such cells appear to be derived from the epithelium. In the liver this derivation is particularly clear since in some of the bile ducts normal-appearing epithelium shares with these large cells in forming the lining mucosa. The presence of an

intact epithelial layer beneath the large cells in some of the renal tubules may be explained either by migration of detached cells or by the regeneration of a new layer to replace those that are damaged. For the present, therefore, these cells must be interpreted as being due to degenerative changes in their respective epithelia. The most unusual feature in regard to them is that in two such dissimilar organs precisely the same type of change should occur.

There seems no doubt that a definite familial tendency exists, since there have been enough reports of either erythroblastosis, congenital hydrops or icterus gravis in several children of the same mother to exclude mere coincidence. The familial occurrence quite properly brings up the question of whether or not the presence of congenital hydrops or erythroblastosis in one or more offspring is sufficient reason for advising against having additional children. Perhaps we are not prepared to answer this in the present state of our knowledge, as it is entirely possible that the lesser degrees of the condition may be treated successfully by transfusions, when recognized early. Reports of such successful therapy have already been made.

Studies, particularly of multiple cases occurring in the offspring of one mother, clearly indicate that congenital hydrops, icterus gravis and erythroblastosis form a group of diseases having a common etiological factor. Congenital hydrops usually results in premature or stillborn infants. Icterus gravis and erythroblastosis may occur in varying degrees and also in combination with each other or with congenital hydrops. It seems quite likely that a number of cases of erythroblastosis have been diagnosed as "hemorrhagic disease of the newborn" and it may also be true that some of the other diseases of the newborn of unknown etiology may belong to this group.

Erythroblastosis is indeed an intriguing subject and since very little is definitely known in regard to it or its etiology, and as additional cases appear, opportunity will be provided for investigative work of value. More complete blood studies of newborn infants with milder degrees of icterus and anemia, as well as in those cases of severe icterus and erythroblastosis, will undoubtedly yield much information in regard to this group of conditions. Such blood studies might well include the Van den Bergh reaction, since it has been suggested that a congenital hypoplasia or partial agenesis of bile ducts may be concerned in the etiology. Lippman¹⁷ has made complete studies of the blood in 71 newborn infants and his work may well

form a basis for comparison. Some work has also been reported on the amount of hemosiderosis in the normal infant, but further investigation of this phase should prove of value.

As far as can be learned, intensive blood studies of siblings of patients having erythroblastosis and of the adult members of these families have not been made. These studies should be undertaken and should include fragility tests of the red blood cells. Now that sickle cell anemia and ovalocytosis have been proved to be familial and hereditary, such studies promise to be of still greater significance.

SUMMARY

1. A case of fetal erythroleucoblastosis with ascites is reported, in which microscopic study revealed certain unusual features.

2. In addition to marked hematopoiesis in liver, spleen, kidney, bone marrow, lymph nodes, thymus, diaphragm, pharynx, small intestine and pancreas, similar foci were found in the chorionic villi.

3. In the lesser bile ducts and in some renal tubules numerous giant cells were found, apparently resulting from alterative changes in damaged epithelium.

4. The generic relationship and probable intrinsic constitutional etiology of congenital hydrops, icterus gravis and fetal erythroleucoblastosis of the newborn are pointed out and suggestions made as to future investigations along these lines.

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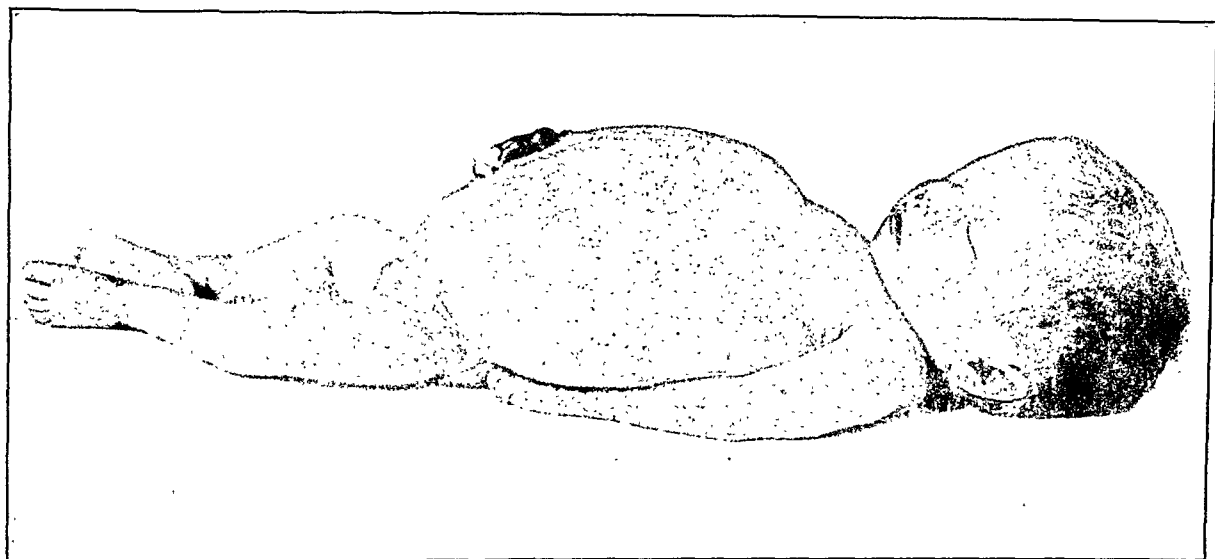
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DESCRIPTION OF PLATES

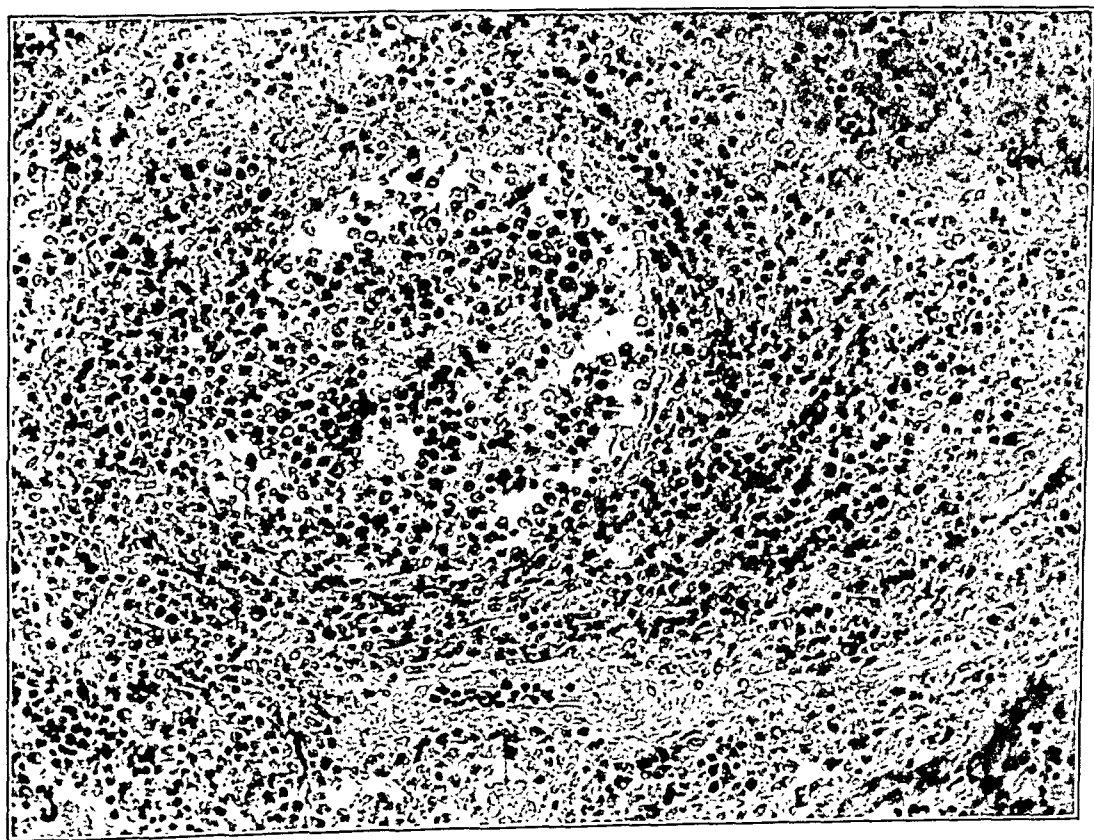
PLATE 102

FIG. 1. Erythroleucoblastosis. Death at age of 6 hours. Extreme ascites. Multiple petechial hemorrhages and areas of pigmentation over abdomen and thighs.

FIG. 2. Marked hematopoiesis in liver in case of erythroleucoblastosis. Follicular arrangement about small bile duct. Hemalum and eosin stain. X 220.



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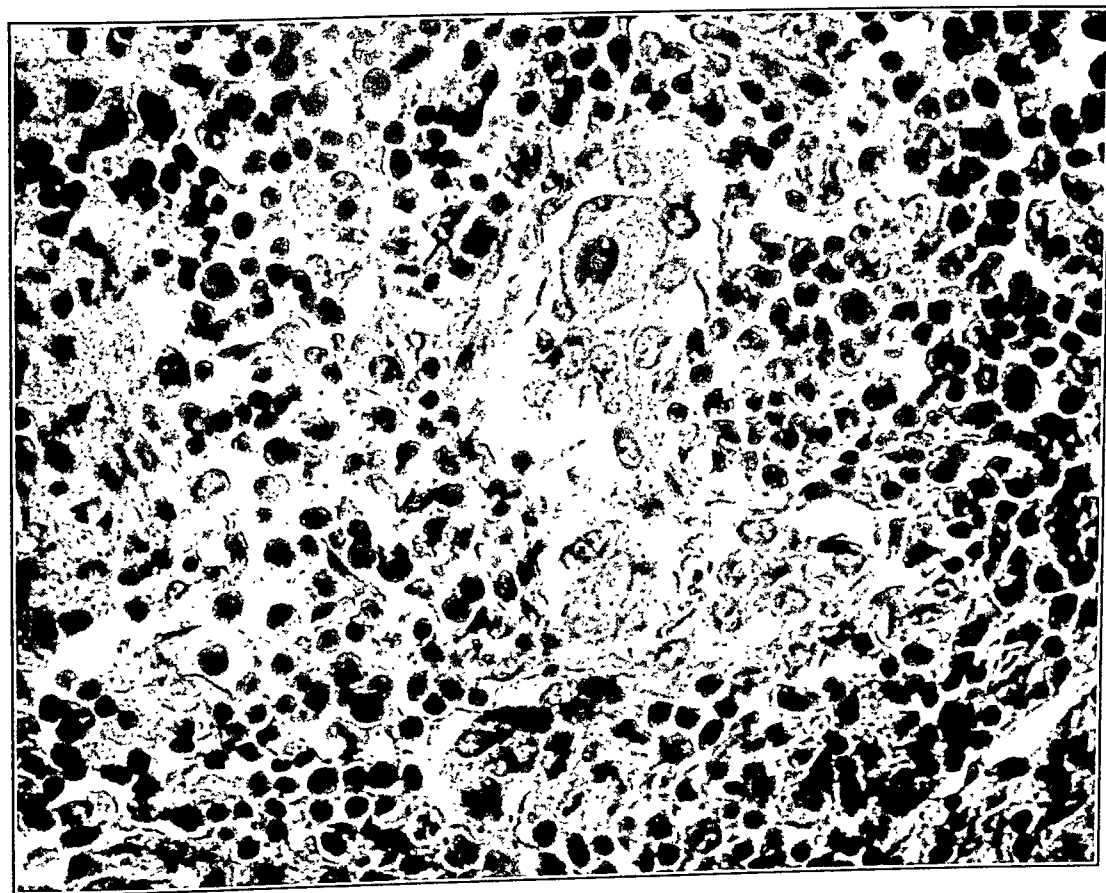
PLATE 103

FIG. 3. Marked hematopoiesis in kidney in same case as Figs. 1 and 2. Follicular arrangement of the leucoblastic tissue. These infiltrations are most marked in the deepest portion of the cortex and in the adjacent medulla. Hemalum and eosin stain. $\times 220$.

FIG. 4. Small bile duct surrounded by hematoblastic tissue and showing in its lumen several mononuclear giant cells, probably derived from the epithelium. Hemalum and eosin stain. $\times 510$.



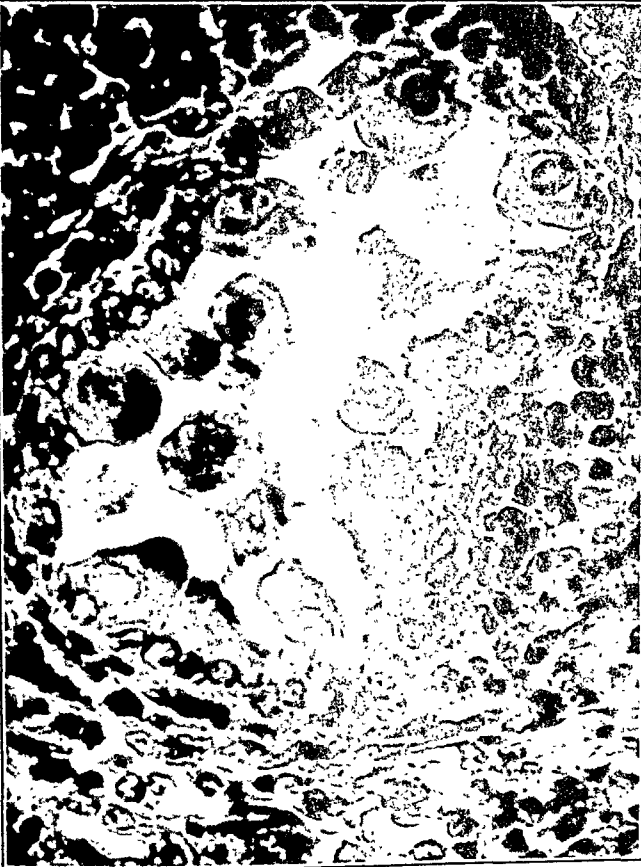
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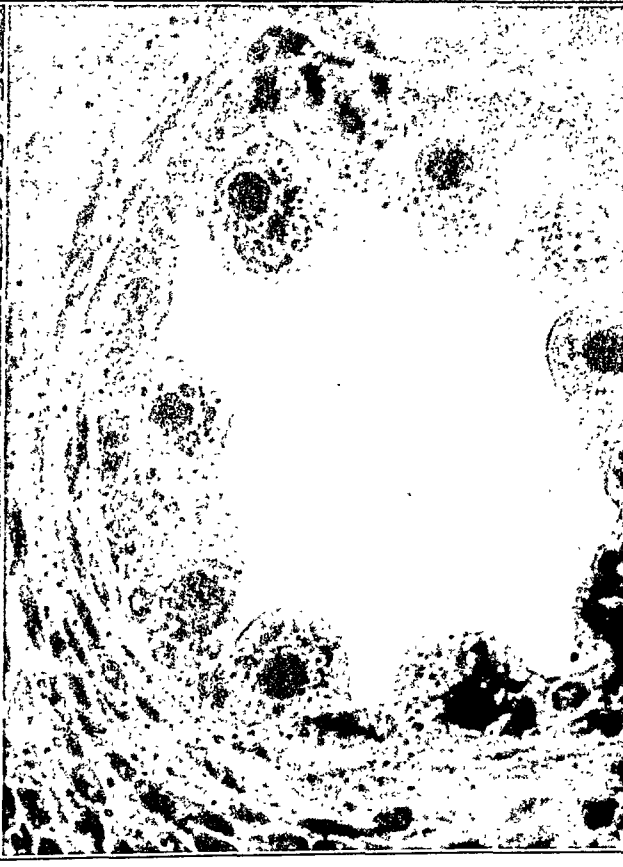
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PLATE 104

- FIG. 5. Renal tubule embedded in hematoblastic tissue. This tubule shows numerous mononuclear giant cells, which in size, cytological detail and relation to the epithelium appear identical with those in the liver, as illustrated in the preceding figure. Hemalum and eosin stain. $\times 510$.
- FIG. 6. Large mononuclear cells in a renal tubule showing the small basic-staining granule lying beside the nucleus, as well as the numerous lipoid droplets within the cytoplasm. Hemalum and Sudan III stain. $\times 510$.
- FIG. 7. Hematopoietic tissue in stems of the central chorionic villi. Hemalum and eosin stain. $\times 220$.



5



6



7

CONGENITAL HEART DISEASE WITH PULMONARY ARTERITIS*
INTERVENTRICULAR SEPTAL DEFECT, DEXTROPOSITION OF
THE AORTA AND DILATATION OF THE PULMONARY ARTERY

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A solitary opening at the base of the interventricular septum in association with other defects ranks as the most frequent cardiac anomaly, the commonest of these combinations being the "Tetralogy of Fallot." A somewhat similar, though much rarer, combination consists of a ventricular septal defect, dextroposition of the aorta, and dilatation of the pulmonary artery instead of the usual stenosis. The first example of this condition was reported by Dalrymple in 1847; in 1897 Eisenmenger¹ presented the first complete study of such a case in which a correct ante mortem diagnosis had been made by his colleague Von Schrotter; and in 1927 Abbott² listed 8 cases, 3 of which she had studied personally. Clinically the condition can be differentiated from the tetralogy by the absence of or only a moderate degree of clubbing and cyanosis, the localization over the precordium of a harsh systolic murmur at the defect, not transmitted into the vessels, the occasional presence of a diastolic murmur of pulmonary insufficiency and the distinctive character of the roentgen-ray picture (Baumgartner and Abbott³). The case here presented illustrates these anomalies in association with inflammatory and degenerative lesions in the pulmonary artery and in its branches. One of the chief interests of this study lies in considering the nature of that arteritis and sclerosis, its etiology and pathogenesis, and the possible mutual relations and effects, both beneficial and deleterious, of the cardiac and arterial lesions on each other.

REPORT OF CASE

Clinical History: R. A., a white male painter, aged 60 years, had always been in good health and a hard worker. For 30 years he drank a pint or more of whiskey daily and led an otherwise dissipated life. He recalled measles and

* Received for publication March 30, 1933.

mumps in childhood but denied syphilis, gonorrhea, scarlet fever, diphtheria, pneumonia and influenza. There was a negative history regarding cyanosis, arthritis, respiratory embarrassments and infections. He was the father of 7 children, 4 of whom died in infancy. His wife had had no miscarriages. Early in the summer of 1931 his present illness began rather suddenly with dyspnea, weakness and fatigue. He stopped work for a few months and took a complete rest in bed.

In November he resumed his occupation and for a time got along fairly well. In December the symptoms returned, aggravated by failing vision, urinary retention, edema, cyanosis, thoracic pain and a persistent productive cough with small hemoptysis on several occasions. In January 1932, for the first time, he consulted a physician. Despite rest and treatment he declined rapidly and progressively and on March 13, 1932 was admitted in a critical condition to the Jefferson Hospital.

Physical Examination: The patient was orthopneic, the skin and mucous membranes deeply cyanosed, and the subcutaneous tissue markedly edematous. The pupils were equal but reacted poorly to light and accommodation. The nails were not curved and the fingers and toes were not clubbed. The temperature was subnormal.

Cardiovascular System: The pulse was 104, regular, full and hard. The jugulars were markedly distended, pulsations being visible in the episternal notch and over the carotids. There was no precordial bulging, the area of cardiac dullness was obliterated, the apex beat was neither visible nor palpable and there was no shock or thrill. The second pulmonic and both aortic sounds were accentuated and a soft blowing murmur was heard over the apex. The blood pressure was 180/120.

Respiratory System: The chest was of the emphysematous type, respirations 26 to the minute, harsh and labored but regular. Fremitus slightly decreased. The percussion note was impaired over both apices and posteriorly over both bases, elsewhere it was hyperresonant. Breath sounds harsh, expiration prolonged. Sibilant and sonorous râles were heard over the entire chest and crepitant râles over both bases and in both axillae.

Abdomen: The spleen was palpable. The edge of the liver was 10 cm. below the last rib.

Morphine and atropine were given, and venesection was performed, 600 cc. of black, thick, viscid, slowly flowing blood being withdrawn. Following this the patient appeared relieved and went to sleep. Six hours later, without any evident clinical change, he emitted one loud gasp and died.

POSTMORTEM EXAMINATION

The autopsy was performed 7 hours postmortem. The skin and mucous membranes were a deep purplish red in color, the lips dry and swollen. The subcutaneous tissue of the abdominal wall, genitals, lower extremities, the peritoneum and the wall of the bowel were markedly edematous. The peritoneal cavity contained a slight excess of fluid and the pericardial cavity approximately 300 cc. of clear, straw-colored fluid. The pleural cavities were without adhesions or fluid.

*Heart** (*Fig. 1-A and 1-B*): The organ was rotated clockwise, the pulmonary artery was somewhat horizontal, the walls of all the chambers were hypertrophied and the left ventricle and both auricles were dilated. The right ventricle was in a condition of simple hypertrophy and the chamber was not much enlarged. It extended downward to within 1.5 cm. of the apex, which was formed entirely by the left ventricle. The musculature contained numerous, firm, grayish yellow streaks and flecks. In the upper part of the ventricular septum there was a large, funnel-shaped defect bounded anteriorly and inferiorly by the thickened muscle of the septum, superiorly by the right aortic cusp and posteriorly by the pars membranacea. It tapered from left to right and opened into the sinus of the right ventricle beneath the septal cusp of the tricuspid valve, which stretched across and screened it much as the infundibular cusp stretches across the pulmonary conus in the normal heart. The septal cusp sent two bundles of chordae tendineae from its ventricular aspect to be inserted into the muscular border of the defect about its middle, and the anterior of these contained three large chordae attached to a small papillary muscle. The wall of the conus was hypertrophied and the lumen somewhat narrowed. The latter appeared to have resulted from the compensatory hypertrophy of the crista supra ventricularis, which formed the right anterior margin of the defect on the side of the right ventricle and screened the opening of the defect from the interior of the conus which opened into a dilated pulmonary artery. The endocardium lining the conus and the defect was thickened and opaque. There were no vegetations about the defect or on the tricuspid leaflets. The pulmonary cusps were irregularly widened, the commissures were broadened and the free margins rolled inward. There were several massive aggregations of calcified material on the anterior and right posterior pulmonary cusps, the left posterior segment being free of calcification. The nodule on the anterior segment occupied the right angle of the sinus of Valsalva and extended also on the ventricular surface of the cusp at that point. It was the size of a bean and consisted of an aggregate of small calcified nodules massed together. The right posterior cusp was thickened along its free mar-

* The following gross description of the heart was corrected and revised by Dr. M. E. Abbott, under whose direction the drawings represented in Figs. 1-A and 1-B were prepared. The heart specimen and microscopic slides of all the organs are filed in the Medical Museum of McGill University, Montreal, Canada, Accession No. E-10589.

gin and had two areas of calcification, one nodular and the other along its line of attachment. Above the cusps the pulmonary artery was dilated, inelastic, finely scarred and irregularly thickened by discrete and confluent atheromatous plaques. The mitral leaflets were atheromatous. The aorta was shifted to the right and overrode the ventricular septal defect so that it appeared to arise from both ventricles. It presented several patches of atheroma and calcification, the largest being 1.2 by 0.7 cm. in size. The coronary arteries appeared to be normal. The foramen ovale was closed and the ductus arteriosus obliterated. The coronary sinus was dilated.

Weights and Measurements: Heart 600 gm., 13 by 13 by 8 cm.; mitral valve 10.5 cm. in circumference; tricuspid valve 12 cm.; wall of conus 3 cm. in thickness; walls of right and left ventricles and of the interventricular septum bordering inferior aspect of ventricular septal defect each measured approximately 2 cm. in thickness; dimensions of left ventricular aspect of ventricular septal defect 1 by 2.5 cm.; circumference of lumen of conus at narrowest point 2.5 cm.; circumference of pulmonary orifice at level of cusps 8 cm., and 2 cm. above this line the circumference was 9 cm.; circumference of aorta at semilunar valves and also 2 cm. above this line 7.5 cm.

Lungs: The pleura was slightly thickened, dark red and wrinkled, and beneath it were many red infarcts, between which the lung tissue was tough and fibrous. The bronchi were thickened and their lumens filled with blood-tinged mucus. The lumens of the pulmonary arteries of the first and second order were partially or completely occluded by red thrombi that were propagated into their subdivisions; otherwise, they presented the same changes noted in the main trunk. The smaller intrapulmonary branches were rigid, concentrically or eccentrically thickened, stood out prominently on the cut surface of the lung and spurted blood when incised.

Weights and Measurements: Left lung 630 gm., 25 by 14 by 8 cm.; right lung 770 gm., 28 by 19 by 7 cm.

Anatomical Diagnoses: Ventricular septal defect, dextroposition of the aorta, dilatation of the pulmonary artery, hypertrophy of the walls of the conus and of both ventricles, pulmonary artery sclerosis and arteritis with thrombus formation, healed calcified endocarditis of the pulmonary semilunar cusps, multiple pulmonary infarcts, generalized passive congestion with edema.

MICROSCOPIC EXAMINATION

In addition to the usual sections pieces of tissue were taken from the pulmonary artery and from several of its branches; from the main bronchi, and from the apices, bases, anterior, posterior and central portions of all lobes of both lungs. They were fixed in dilute formalin and Zenker's fluid, embedded in paraffin and cut and stained with hematoxylin and eosin, Verhoeff's elastic tissue stain, Mallory's and Van Gieson's connective tissue stains, Weigert's fibrin stain, Gram's stain for bacteria in tissues and Levaditi's stain for spirochetes.

Many of the extrapulmonary and large intrapulmonary branches are thrombosed (Fig. 2). The intima is retracted by underlying scars, irregularly destroyed and much thickened by atheromatous, calcified, fibrous and hyalinized plaques that penetrate deeply to replace large portions of the media. The internal elastica is split, frayed, reduplicated and in places much reduced. The intima contains a few small plaques consisting of necrotic cells with distorted and elongated nuclei intersprinkled with lymphocytes, granular leukocytes and multinucleated cells. In the adventitia there are nodular and linear collections of lymphocytes, plasma cells and proliferating fibroblasts about the vasa vasorum, which are markedly thickened (Fig. 3). This inflammatory tissue projects fanwise into the media to form stellate scars that interrupt and replace the muscularis and elastica (Figs. 4 and 5). A somewhat similar inflammatory process is observed in the tissue surrounding the arteries. The walls of the intrapulmonary arteries measure 1 to 3 mm. in diameter (Fig. 6) and are devoid of inflammatory cells, the vasa vasorum are thickened, and the muscularis of the media is largely replaced by an increase in fine and coarse collagen and distorted elastic fibrils. The internal elastica is hypertrophied, split, frayed and reduplicated. The lumens are partially occluded by fresh and organized thrombi. Atheroma and calcification are rarely encountered. The perivascular granulation tissue is prominent. The intrapulmonary arteries below 0.5 mm. (Fig. 7) seem fewer in number but proportionately thicker than normal, and the muscularis is largely replaced by connective tissue. The internal and external elastica are distorted and occasionally fused. There are fresh, organized and canalized thrombi, and hyalinized papillary nodules project into the lumen. Most of

the walls are free of inflammatory cells but a few are diffusely infiltrated by lymphocytes and endothelial leukocytes. With the decrease in the size of the arteries, the perivascular granulation tissue becomes correspondingly more prominent. It varies from active inflammatory nodules to dense hyaline, linear and stellate scars that stretch at length through the pulmonary parenchyma. The capillaries and small veins appear dilated, reduced in number, thickened and hyalinized. Some of the larger veins are thrombosed. The pulmonary alveoli vary in size, many are enlarged and their walls thin and ruptured. In other areas the septa are fibrosed and thickened, and contain interstitial vascular nodules consisting of lymphocytes, plasma cells and fibroblasts that invade and obliterate the air spaces (Fig. 8). Infarcts are numerous, hemorrhage extensive, heart-failure cells plentiful. Anthracosis is minimal. The epithelium of the bronchi is hyperplastic and in many places denuded. The walls are thickened by vascular collections of lymphocytes and fibroblasts which occasionally penetrate through the wall to invade the lumens. There is a subintimal thickening of the bronchial arteries and a chronic proliferative reaction in the peribronchial tissue. The pleura is diffusely thickened by hyalinized and cellular connective tissue containing small, discrete, vascular lymphocytic foci. The aorta contains degenerative changes of the nature of atherosclerosis, the media is somewhat hypoplastic and the vasa vasorum are slightly thickened. There are submiliary nodules consisting of sparse infiltrations of lymphocytes, plasma cells and endothelial leukocytes about a few newly formed capillaries in the media. In the myocardium are many irregular, loose, nodular, cellular collections consisting mainly of endothelial leukocytes, proliferating fibroblasts and necrotic muscle fibers. Intermediate stages of healing can be traced up to dense hyalinized scars that frequently contain one or more blood vessels in the center.

Spirochetes or other organisms are not demonstrated in any of the tissues.

DISCUSSION

The Cardiac Defect: With the combination of ventricular septal defect and dextroposition of the aorta, without associated pulmonary stenosis, the average life is 16 years; the highest age attained heretofore is 33 years. The patient whose case is under discussion lived to

be 60. Because of his critical condition the physical examination was necessarily superficial. He was in the hospital only 8 hours when death occurred, so that careful studies were not carried out and a positive ante mortem diagnosis was not made. As the anatomical conditions in the heart favored oxygenation, the admixture of venous with arterial blood was negligible, which accounts for the absence of clubbing and until the last illness the absence of dyspnea and cyanosis. The relatively high pressure in the left ventricle, the healed pulmonary valvulitis, the contour of the ventricular septal defect, the patches of fibrosis on the endocardium bordering the defect and on the wall of the conus opposite support this assumption. The ventricular septal defect probably actually contributed to longevity by permitting the force of both ventricles to combine in propelling blood through the lesser circuit against the resistance offered by the sclerosed pulmonary arteries. Dyspnea and cyanosis were of course coincidental with the onset of myocardial insufficiency, when in all probability the shunt through the defect became venous-arterial. It has been considered in these cases that the pulmonary artery undergoes dilatation to accommodate the additional volume of blood received from the left ventricle through the defect into the pulmonary circulation. In our case it is quite possible that it was due in part to the arteritis (Warthin ⁴). In Blechmann and Paulin's case,⁵ the dilatation was attributed to a streptococcic pulmonary endarteritis.

An inflammatory arteritis of the pulmonary arteries and arterioles complicating congenital heart disease is exceedingly rare (Abbott ²). The nature of the lesions in our case rests mainly on their anatomical and histological appearances and involves a consideration of syphilis, atherosclerosis, hyperplastic arteriosclerosis and rheumatic arteritis.

The characteristics of syphilitic pulmonary artery sclerosis and arteritis have been well established (Cheney,⁶ Hare and Ross,⁷ Konstam and Turnbull,⁸ Karsner *) by the occurrence of pulmonary arterial aneurysms and the demonstration of spirochetes in the walls (Warthin and coworkers,^{9, 10}); by significant lesions of the pleura,

* Since submitting this paper for publication the following article has come to our attention.

Karsner, H. T. Productive-cicatricial syphilitic disease of the pulmonary artery. *Arch. Int. Med.*, 1933, 51, 367-386.

bronchi and pulmonary parenchyma (Landsberg,¹¹ McIntyre¹²); positive Wassermann and Kahn reactions on the blood serum of patients (Winternitz and Schmeisser¹³), and the finding as associated conditions paresis, tabes, aortitis, chronic orchitis and visceral gummas. Although the evidence in our case is incomplete from the clinical aspect, the prevailing character of the inflammatory reaction simulates more that of syphilis than of any other type of lesion. It is possible that all the lesions may represent the result of the fetal syphilis that ranks high as an etiological factor in congenital heart disease. This is strengthened by the character of the myocardial lesions (Abbott²). Some authors (Warthin¹⁴ and Boyd¹⁵) have described a syphilitic type of myocarditis that they regard as being quite characteristic of the disease. We are not in accord with their opinion but agree with Saphir,¹⁶ who believes that no myocardial lesion can be used as a diagnostic criterion of acquired syphilis. In a rather extensive search of the literature we have encountered no proved case of syphilitic pulmonary arteritis complicating congenital heart disease. Arrillaga's speculations in this connection¹⁷ are not well supported and await confirmation.

A degenerative atherosclerosis develops in the larger branches of the pulmonary arteries secondary to a variety of congenital and acquired lesions of the heart, lungs, pericardium, pleura and blood vessels (Miller¹⁸). It is not uncommonly observed in association with defects at the upper part of the interauricular septum, in which case such a large influx of blood is sent into the pulmonary circulation that it creates a strain on the arterial walls. It is rarely seen with the type of defect dealt with here and never to so marked a degree (Abbott^{*}). We consider that it was probably engrafted on the inflammatory process mainly as a secondary manifestation, which is not uncommon in syphilitic aortitis. The chronic alcoholism and possible lead poisoning (see occupation) might have contributed to it (Karsner¹⁹), although the opinion that lead can cause arteriosclerosis is founded on autopsy material in lead workers (Aub and coworkers²⁰) and is without experimental support (Ophüls²¹).

A condition of chronic cyanosis, erythrocytosis, and dyspnea occasionally results from hyperplastic sclerosis of the finer pulmonary arterial radicles. Histologically it is characterized by proliferation and reduplication of the internal elastic lamina and a

* Personal communication.

corresponding atrophy and fibrosis of the media (Bacon and Apfelbach²² and Rosenthal²³). The pleura, bronchi, pulmonary parenchyma and walls of the pulmonary arteries are devoid of inflammatory cells. Such a case has recently come under our observation (Sokoloff and Stewart²⁴) and except for a few of the changes in the smaller arteries we find the lesion entirely unlike the one dealt with in the present communication.

A more or less characteristic inflammatory reaction, varying somewhat with the stage of the disease (Swift²⁵) occurs in the pulmonary artery (Paul,²⁶ Kugel and Epstein²⁷), pulmonary parenchyma (Rabinowitz,²⁸ Naish,²⁹ Sacks,³⁰ Eiman and Gouley³¹), pleura (Paul³²), and bronchi (Fraser³³) in certain persons suffering from rheumatic fever. The changes affect the smaller pulmonary arterial twigs and consist of fibrin, erythrocytes and multinucleated cells in the muscularis and immediately beneath the endothelium. The internal elastic lamina becomes frayed and permanently lost. Surrounding the affected vessels are fibrin, erythrocytes, granulocytes in great numbers, radially arranged cells with bizarre nuclei, and beyond these a few plasma cells and dilated capillaries. Healing is specific, thrombi are never encountered and the extrapulmonary branches are uninvolved (Pappenheimer and VonGlahn^{34, 35}). In our case a few small arteries presented lesions resembling somewhat those representative of the healing stage. However, the prevailing character of the active perivascular, pleural, bronchial and pulmonary parenchymal granulation tissue, the inflammation and extensive thrombosis of the extrapulmonary arteries were more illustrative of syphilis than of rheumatic fever. The viscera were carefully and critically scrutinized for evidence of rheumatic lesions. The nodules on the pulmonary cusps were suggestive, but similar active, non-rheumatic vegetations have been noted before in congenital heart disease (Moschowitz³⁶). The access of arterial blood to the right heart is thought to be in some way the determining factor (Boldero and Bedford³⁷). Sections selected from the free margins and bases of the pulmonary cusps and from the musculo-arterial junction failed to reveal rheumatic lesions (Kugel and Epstein²⁷). The vast majority of the myocardial lesions were non-specific and may well have been vascular in origin. A few resembled Aschoff nodules which VonGlahn and Wilshusen³⁸ observed in the myocardium of subjects whose aortas presented characteristic, well advanced lesions of

syphilitic arteritis. The mild inflammatory changes in the aorta of our case were not distinctive and might possibly have been due to rheumatic fever (Pappenheimer and VonGlahn^{34, 35}) or to latent syphilis. Hare and Ross⁷ found the aorta uninvolved in 8 of 16 unquestioned cases of syphilis of the pulmonary artery.

SUMMARY AND CONCLUSIONS

The clinical, anatomical and histological features of a case of pulmonary artery sclerosis and arteritis associated with an interventricular septal defect, dextroposition of the aorta and dilatation of the pulmonary artery are presented and discussed. There were no symptoms until the last few months of life and death occurred at the age of 60 years, the highest ever attained by an individual with this combination of congenital lesions.

The shunt through the defect in the ventricular septum was probably arterio-venous; this permitted the combined force of both ventricles to unite in overcoming the resistance offered by the sclerotic lesser circuit.

The evidence adduced, although not empirical, strongly favors a syphilitic origin for the lesions in the pulmonary artery, the pulmonary parenchyma, the pleura and bronchi.

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DESCRIPTION OF PLATES

PLATE 105

FIG. 1-A. Anterior view of the heart illustrating marked hypertrophy of the crista supraventricularis (beneath which the defect opens) and of the wall of the right ventricle and conus, the lumen of which is narrowed. Note the dilatation, scarring and sclerosis of the pulmonary artery, the thickening of the cusps and the widening of the commissures.

FIG. 1-B. Posterior view of the heart illustrating the large funnel-shaped defect in the upper part of the interventricular septum, just beneath the right aortic cusp. Note the dextroposition and slight atheroma of the aorta. The incised myocardium contains numerous fine, grayish flecks and streaks.

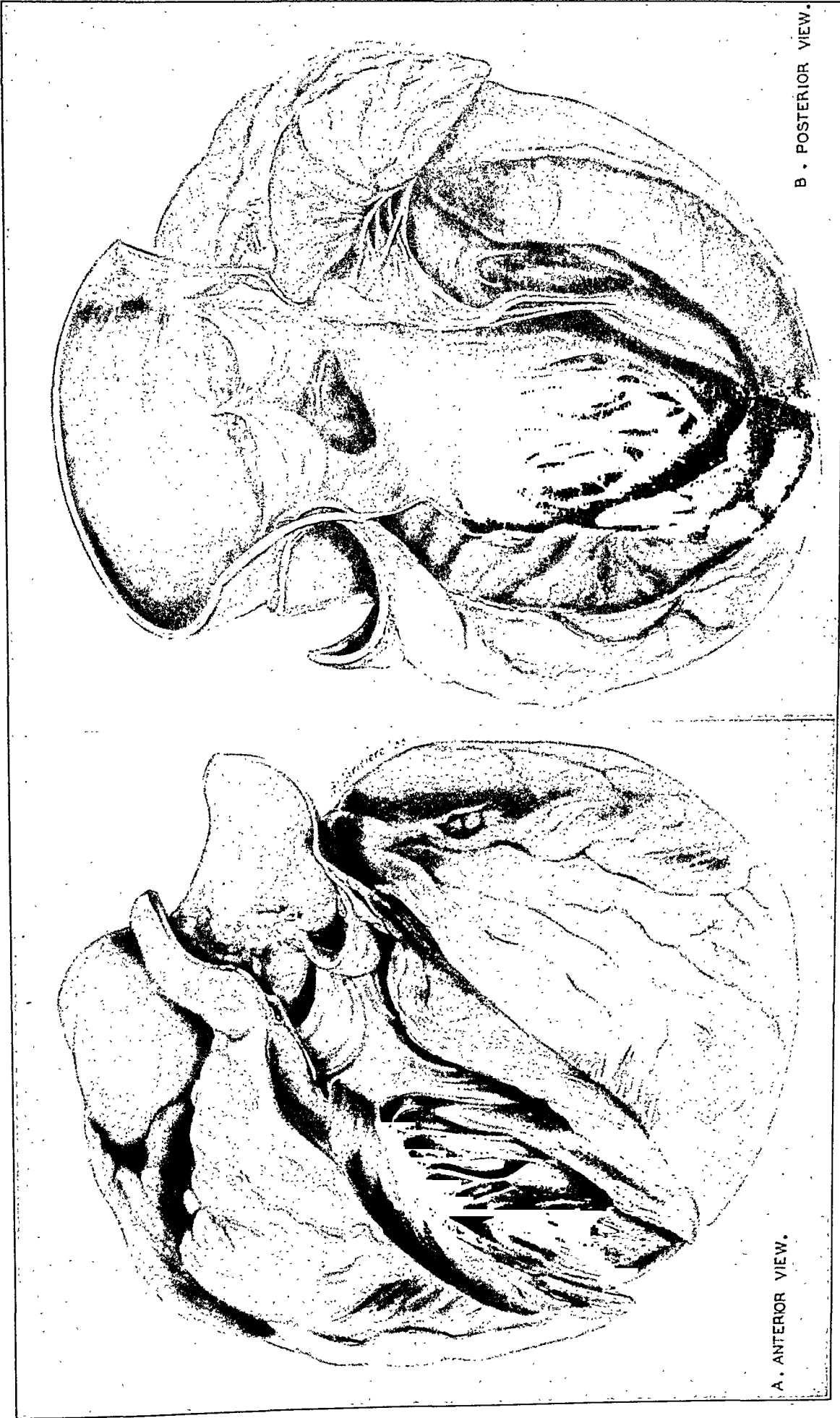
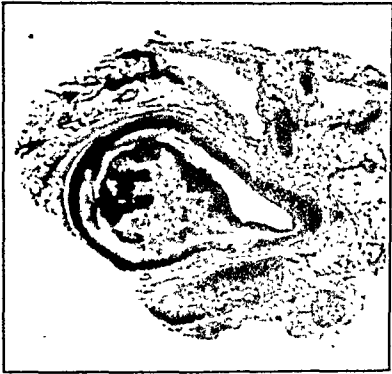


PLATE 106

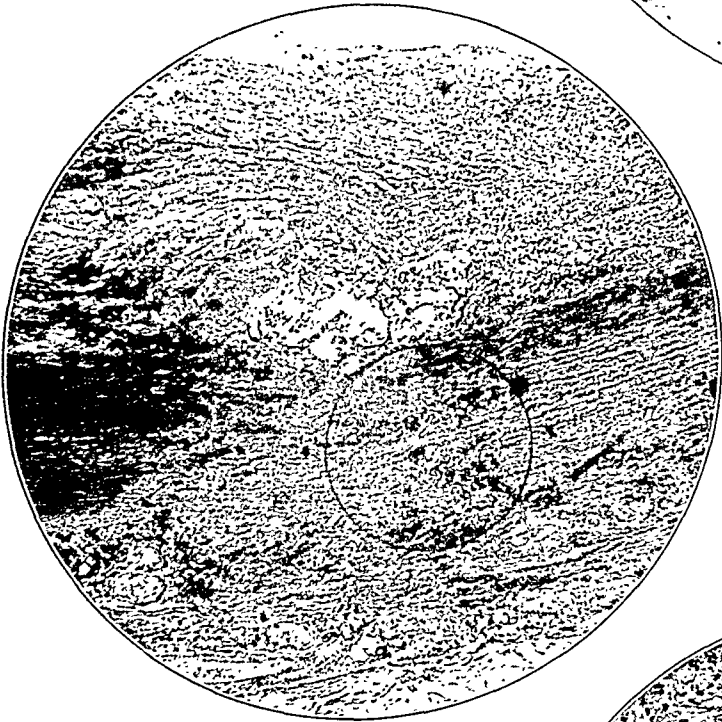
- FIG. 2. Large intrapulmonary branch of the pulmonary artery with thrombus formation, perivascular fibrosis and marked destruction of the wall. Photograph of histological slide, $1\frac{3}{4}$ times actual size. Elastic tissue stain.
- FIG. 3. Round cell infiltration of the vasa vasorum of the pulmonary artery extending into the media. The intima is thickened by hyalinized and atheromatous plaques. $\times 57$.
- FIG. 4. Wall of the pulmonary artery where the inflammatory changes are similar to those observed in Fig. 3. The involvement of the media is more extensive. $\times 35$.
- FIG. 5. Inflammatory reaction in the portion of the media contained within the circle in Fig. 4. Note the leukocytic infiltration, newly formed vessels and fibrosis. $\times 115$.



2



3



4



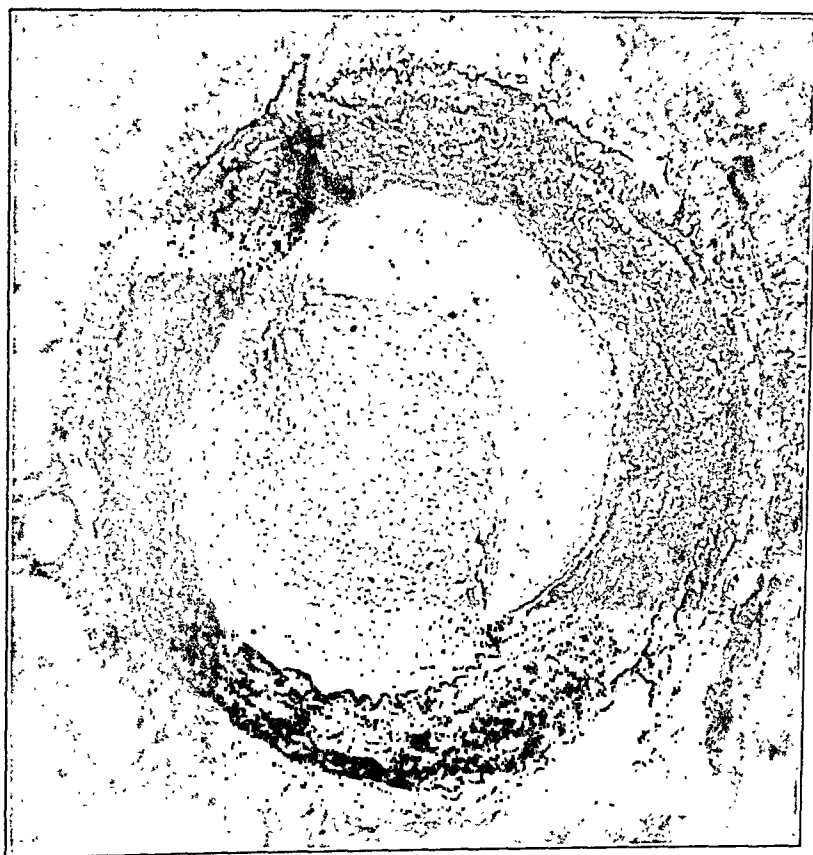
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PLATE 107

FIG. 6. A branch of the pulmonary artery illustrating detailed changes in elastic fibers; the lumen is partially occluded by an organized thrombus. Elastic tissue stain. $\times 85$.

FIG. 7. A small branch of the pulmonary artery with marked obliterative endarteritis and perivascular fibrosis. $\times 130$.

FIG. 8. An interstitial pulmonary inflammatory nodule forming an eccentric collar about a small thickened branch of the pulmonary artery. The nodule is markedly vascular and consists of lymphocytes, plasma cells, endothelial leukocytes and proliferating fibroblasts. $\times 100$.



6



7



8

A REDUCING SUBSTANCE FOUND IN CHROMOPHILIC ADENOMAS AND IN THE NORMAL ANTERIOR PITUITARY *

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The present investigation was prompted by a chance observation. When examining an operative specimen from a patient with active acromegaly a smear of the tumor (a typical acidophilic adenoma) was stained with methylene blue. The cells were deeply stained and their histological characteristics well displayed at first, but while the slide was being examined all traces of blue color rapidly disappeared. Other smears were stained and the same slide restained, with always the same result: the smallest fragment of tumor tissue was able to decolorize many times its volume of approximately 1 per cent methylene blue within fifteen minutes. Each time the color returned on exposure to air. Unfortunately we have not had further opportunity to confirm this single observation.

Although many surviving tissues have the property of reducing methylene blue, none that we have encountered can compare with this particular tumor tissue in its activity. A chromophobe adenoma of the anterior lobe offered the most interesting comparison. Smears from fresh tissue removed at operation in precisely the same way retained color imparted to them by the same solution for over twenty-four hours.

Fresh pieces of normal beef anterior pituitary reduce methylene blue much more rapidly than do neighboring structures, a fact noticed by Dandy¹ when he attempted a vital staining of the gland *in situ*. But the reducing power of anterior lobe tissue is not strikingly greater than that of liver, muscle and certain other tissues that have been studied so thoroughly by Thunberg² and his followers.

Further investigation showed, however, that the mechanism of reduction is quite different from that of the hydrogen transportase system studied by Thunberg. In the first place the reaction is proportionately accelerated by successive increases in temperature up to 100° C, and this fact indicates that the substance involved is

* Received for publication May 25, 1933.

probably an actual reducing agent in itself, rather than an enzyme-substrate system. It does not, however, reduce Benedict's solution or give the other common tests for sugar.

In the second place the reducing substance may be extracted from fresh glands by weakly alkaline solutions and, if filtered or centrifuged off, may be kept for weeks in the cold as an opalescent protein solution. If the protein is precipitated by the ordinary methods (sodium sulphate, alcohol, acidification) most but not necessarily all of the reducing substance is carried down with it.

The question naturally at once arises, is the reducing substance related to any of the physiologically active principles of the anterior hypophysis? The characteristic constituent, and probably the quantitatively predominant one, in the acidophilic adenoma is the growth-promoting principle, while any constituents that such a tumor may have in common with a chromophobe adenoma may apparently be ruled out as a source of the reducing substance. The growth-promoting principle derived from normal anterior lobes clings stubbornly to the globulin fraction, and so does the reducing substance. On the other hand, the reducing substance survives many procedures (such as boiling) that are known to be fatal to the growth principle. Tests on immature female rats show that the reducing substance is not identical with the estrus-producing substance.

Further studies on methods of quantitating the reducing substance on its chemical nature and on its relation to the growth principle are being carried on by Dr. William Salter and Dr. Arda Green.

SUMMARY

A substance reducing methylene blue has been demonstrated in the normal anterior pituitary and its extract, and in larger amounts in a chromophile adenoma. None was found in a chromophobe adenoma.

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THE EFFECT OF TESTICULAR EXTRACT ON EXPERIMENTAL TUBERCULOSIS IN RABBITS. I. SKIN LESIONS *

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Duran-Reynals^{1, 2, 3} has shown that there exists in the testes of normal rabbits a substance that has the remarkable power of enhancing skin infections produced by vaccine virus and by staphylococci. Hoffman⁴ confirmed the observation on the enhancement of vaccine virus and extended the work to show that this phenomenon is equally effective with the viruses of vesicular stomatitis, Borna disease, and herpes; and in the case of the viruses of herpes, Borna disease, and vaccinia, the enhancement applied also to central nervous system infections. Pijoan⁵ reported that this substance, when added to cultures of twenty different bacteria, caused enhancement to a high degree of the resulting intradermal lesions. Hoffman and Duran-Reynals⁶ found that the Berkefeld filtrate of testicular extract was equally as effective as the unfiltered emulsion. To this enhancing substance Ledingham and Barratt⁷ have given the name "Reynals factor."

Since all the bacteria used in the above mentioned experiments were those that usually produce a more or less uniform, acute reaction, it was felt that it might be of interest to apply this enhancing substance to an organism giving an entirely different type of reaction, *i.e.*, to the tubercle bacillus. Consequently experiments were designed to determine what effect, if any, testicular extract might have upon the character of the lesions resulting from the intradermal injection of tubercle bacilli in rabbits.

METHODS AND MATERIALS

The tubercle bacilli used in these experiments consisted of avian, human, and bovine strains. The organisms were grown on a modified egg medium † and were from 1 to 2 months old at the time of

* Received for publication April 3, 1932.

† The modified egg medium is prepared as follows:

Potatoes	250 gm.
Distilled water	500 cc.
Glycerin	20 cc.

Autoclave 30 minutes under 15 lb. pressure.

To 50 cc. of the above potato glycerin extract add 50 cc. bouillon and 5 eggs. Put in tubes and inspissate. Add 1 cc. distilled water to each tube.

Autoclave 20 minutes under 15 lb. pressure.

use. A heavy, fresh saline emulsion of the organisms was used for inoculation.

Testicular extract was freshly prepared from testes of healthy adult rabbits as follows. The testes were removed aseptically,* and after the fat was dissected away they were weighed, minced and ground with sand in a sterile mortar. Sufficient Locke's solution was added to make a 1:10 suspension and the mass was then centrifuged at high speed for 15 minutes. The supernatant fluid was passed through a Berkefeld Y filter. This filtrate constituted the extract used in the experiments and it was tested for sterility before use.

Injection masses for each of the avian, human, and bovine strains were prepared for inoculation as follows. The saline suspension of the organisms was divided into two equal parts. To the first part was added an equal volume of testicular extract, and to the second, an equal volume of sterile normal saline. Thus the first mass served as the experimental material and the second as its control. For each strain of organism the injection masses contained approximately the same number of organisms and differed from each other only in that the experimental mass contained testicular extract, whereas the control did not.

Nine healthy, young adult rabbits were used in the experiments. The flanks were clipped and then shaved. The animals were divided into three groups of three animals to each group. One group was used for the avian, one for the human, and one for the bovine strains.

For the experiment in which the avian strain was employed 1 cc. of the experimental injection mass containing a saline suspension of organisms plus an equal volume of testicular extract was injected intradermally in one area, and 1 cc. of the corresponding control was injected in like manner in another part of the shaved skin on the same side. Two animals were thus inoculated. The third received similar injections, with the exception that both sides of the animal were inoculated.

The three animals receiving the bovine strain were inoculated in similar fashion with two control and two experimental injections on either side.

The remaining three rabbits received four inoculations consisting of 1 cc. of the testicular extract-bacilli suspension (human strain)

* All operations on animals were carried out under full ether anesthesia.

on each side, and likewise four inoculations of saline-bacilli emulsion. Thus each animal received a total of sixteen inoculations. The animals were kept in cages and fed the usual laboratory diet.

Twelve to eighteen days were allowed for the disease to develop, during which time the size and character of the lesions were noted from day to day. The animals were then killed and tissue from both the experimental and the control lesions was removed and fixed in a mixture of alcohol and formalin (9 parts alcohol and 1 part formalin). Celloidin sections were cut and stained by the carbol fuchsin method of Ziehl-Neelson.

It was noted at the time of the inoculations that all injections containing testicular extract diffused so rapidly that no wheal was visible at the end of 2 minutes, whereas those injections without testicular extract resulted in wheals that were visible as long as 30 minutes after inoculation.

RESULTS

The lesions resulting from the injection of organisms with testicular extract were without exception more severe and much more extensive than the respective control lesions. This difference was found to be constant, irrespective of the type of organism used. All early lesions in every series were similar in appearance, but as the disease progressed, those resulting from the inoculation of bacilli with testicular extract became more extensive, showed necrosis and ulcerated earlier than the corresponding control lesions.

The lesions resulting from the inoculation of avian or bovine bacilli were more extensive and more severe than those resulting from the injection of the human strain in both the control and experimental injections.

The microscopic changes consisted of a marked infiltration with lymphocytes and monocytes, with occasional tubercle formation. There were also varying degrees of necrosis in some instances. Acid-fast bacilli (occurring singly or in clumps) were present in all lesions. There was no essential qualitative microscopic difference in either the avian, human or bovine lesions and their respective controls.

In order to express the enhancement phenomenon in more concrete terms the average plane area of the lesions in every series was computed. Those due to the avian bacilli plus saline averaged

TABLE I

Avian Strain

Rabbit	Inoculation		Duration	Size and character of lesions	
	Equal parts emulsion plus saline	Equal parts emulsion plus testicular extract		Emulsion plus saline	Emulsion plus testicular extract
A	cc. 1	cc. 1	days 12	cm. 3 x 3	cm. 15 x 4 Marked ulceration
B	1	1	12	4 x 4 Ulcerated	12 x 6 Marked ulceration
C	1	1	13	3 x 2.5	6 x 3.5
C	1	1	13	3 x 2	6 x 3 Ulcerated

TABLE II

Bovine Strain

Rabbit	Inoculation		Duration	Size and character of lesions	
	Equal parts emulsion plus saline	Equal parts emulsion plus testicular extract		Emulsion plus saline	Emulsion plus testicular extract
D	cc. 1	cc. 1	days 15	cm. 3 x 3 Ulcerated	cm. 12 x 6 Marked ulceration
D	1	1	15	3 x 2.5 Ulcerated	8 x 3.5 Ulcerated
E	1	1	16	3 x 1.5	4.5 x 4 Moderate ulceration
E	1	1	16	2.5 x 1.5 Slight ulceration	5 x 3 Moderate ulceration
F	1	1	16	1.5 x 1.5	4.5 x 4 Slight ulceration
F	1	1	16	1.5 x 1	4 x 2.5 Slight ulceration

9.6 sq. cm. in diameter, whereas those following injection of the bacilli-testicular extract mass averaged 42.7 sq. cm. Thus, the latter lesions were 4.3 times the size of their controls. With the bovine

TABLE III
Human Strain

Rabbit	Site for injection	Inoculation				Duration	Size and character of lesions	
		Equal parts emulsion plus saline		Equal parts emulsion plus testicular extract			Emulsion plus saline	Emulsion plus testicular extract
		No.	Amt. in cc.	No.	Amt. in cc.	days	cm.	cm.
G ...	Right side	4	1	4	1	14	Separate nodules about 1.3 x 1.3 Slightly ulcerated	Confluent lesion 9 x 3.5 Moderate ulceration
G ...	Left side	4	1	4	1	14	Separate nodules about 1.2 x 1.2 Slight ulceration	Confluent in denoted lesion 9.5 x 3.2 Moderate ulceration
H ...	Right side	4	1	4	1	14	Separate nodules about 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9 x 3.5 Marked ulceration
H ...	Left side	4	1	4	1	14	Separate nodules about 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9 x 4 Marked ulceration meeting in mid-line
I ...	Right side	4	1	4	1	18	Separate nodules 1.5 x 1 Slight ulceration in centers	Confluent lesion 8.5 x 3.5 Moderate ulceration
I ...	Left side	4	1	4	1	18	Separate nodules 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9.2 x 3.4 Moderate ulceration

strain the controls averaged 4.75 sq. cm., while those resulting from testicular extract injection were 28.6 sq. cm. The latter were therefore 5.6 times the controls in size. The lesions due to human bacilli were more difficult to measure with any reasonable degree of accu-

racy, because a large number of inoculations were made in a small space and became confluent. However, as accurately as could be determined, the control lesions averaged 1.82 sq. cm. in area, while the experimental lesions averaged 7.875 sq. cm. The size of the latter was 4.3 times that of the controls.

The results of our experiments are represented in tabular form (see Tables I, II and III).

DISCUSSION

Considerable evidence has been presented in the literature in support of the fact that testicular extract will increase the severity and extent of acute lesions resulting from pyogenic bacteria or viruses.¹⁻⁵ Our results clearly demonstrate that testicular extract is equally effective in enhancing the lesions resulting from the intradermal injection of avian, bovine or human strains of tubercle bacilli in rabbits. These organisms normally produce lesions that differ from those previously reported in that the incubation period is longer and the reaction is of an entirely different type. Thus, the enhancing effect of testicular extract in infections appears to be general, whether such infections be acute or chronic, bacterial or viral.

It is well known that the avian and bovine strains are much more virulent for rabbits than the human strain. We have found this same relative difference in the severity of the resulting skin infections due to these organisms. Regardless of the size of the control lesions those resulting from the injection of bacilli together with testicular extract were approximately five times as extensive. From these observations and the work of others, it appears conclusive that the extract of normal rabbit testes possesses some property of enhancing the severity of representative lesions from all classes of infections.

It was noted at the time of inoculations that the injection masses containing testicular extract diffused very rapidly, which is in accord with the observations of Hoffman and Duran-Reynals⁶ and of McClean.⁸ It is possible that the resulting enhancement of the infections may be due either to some action of the active principle of testicular extract on the organisms or on the host or both. It seems impossible to say on the basis of our observations whether the enhancement is due to a wider distribution of organisms, an increase in virulence, a decreased resistance of the host, or a combination of these factors. The reader is referred to papers by Hoffman, Duran-

Reynals, and McClean for a discussion regarding the mechanism of action of testicular extract.

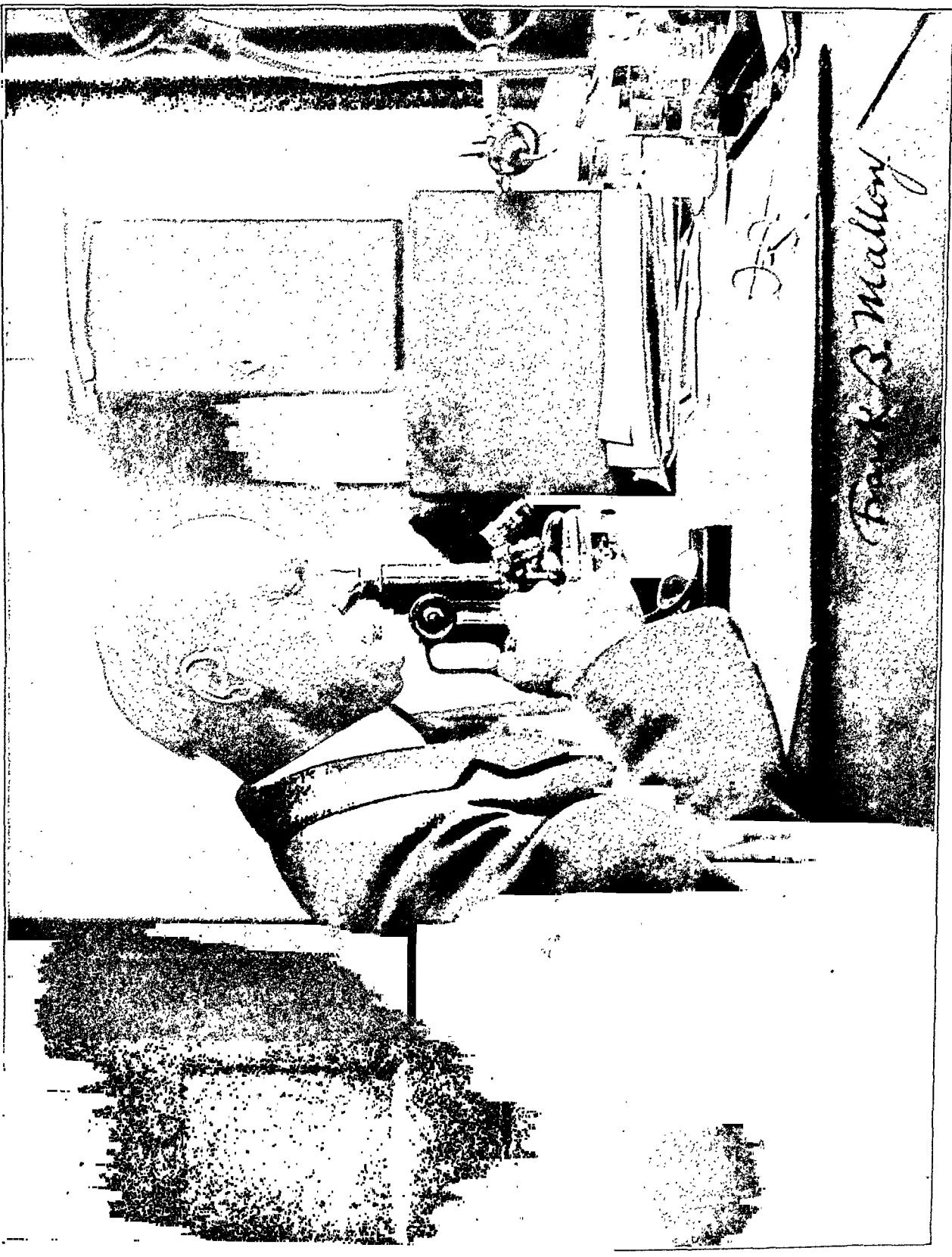
The results of these experiments suggest the possibility of employing this enhancing factor in a clinical connection in animal inoculation of body fluids and exudates where such a procedure is necessary to make a diagnosis of tuberculosis.

CONCLUSIONS

1. When tubercle bacilli together with testicular extract are injected into the skin of rabbits marked enhancement of the resulting lesions occurs.
2. This enhancement takes place equally well with the human, bovine, and avian strains.
3. The practical application of this enhancement phenomenon in laboratory diagnosis is worthy of consideration.

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FRANK BURR MALLORY AND THE PATHOLOGICAL DEPARTMENT OF THE BOSTON CITY HOSPITAL *

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The history of a successful institution is often the history of a man. This is particularly true of the pathological department of the Boston City Hospital.

The hospital was dedicated and opened in 1864 for the treatment of acute and chronic diseases. Dr. Charles E. Swan is referred to as pathologist in connection with the dedicatory exercises, but is not mentioned in the annual report for 1864. Like many of his successors Dr. Swan evidently used the position as a stepping-stone to appointment on the clinical staff, becoming physician to outpatients in 1868. Dr. S. G. Webber was appointed pathologist in 1870 and Dr. William P. Bowles in 1873.

The dead house and autopsy room were located on the east side of Albany Street in a part of the boiler house, near the location of the new Mallory Institute. Complaint was made in 1870, "now that Albany Street has become so great and important a thoroughfare," that the carrying of bodies across the street, not at first objectionable, had become so. In 1871 improvements were made in the boiler house and a morgue was established, not only for those dying in the hospital, but also "for the reception and identification of the unknown dead found elsewhere." Record is especially made in 1878 of the appointment of Dr. E. G. Cutler as pathologist.

In 1880 it is interesting to read that "through the liberality of the City Government the Superintendent's office at the hospital has been connected by telephone with police headquarters."

In 1881 Dr. W. W. Gannett was appointed pathologist with

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Dr. Cutler, who resigned during the next year. Dr. Gannett remained with the institution as pathologist up to 1891. Although he was the most permanent of the pathologists up to this time, he carried on in addition an extensive private and consulting practice, together with teaching.

In the meantime, as the hospital population grew and the number of deaths increased, the necessity of carrying bodies across Albany Street became so objectionable that the morgue and autopsy room were moved to the main hospital grounds. The autopsy room was located on the second floor of the laundry building. In the early days Louis A. Pasco, who served the department as keeper of the mortuary for forty years before retiring, was also employed in the laundry. One of my earliest memories of the hospital is the picture of Pasco carrying a body on his shoulder up the long flight of stairs to the autopsy room. When the autopsy began Pasco's principal duty was to wave a newspaper or fan to keep the flies from Dr. Gannett's bald head.

It is soberly recorded in the report for 1886, after a long discussion, that women medical students were to be admitted to the amphitheater of the hospital, and a petition that homeopathic treatment might be furnished for those who preferred it was denied.

With the establishment of the South (contagious) department there was needed a laboratory for the diagnosis of diphtheria, and a long narrow room was supplied on the basement floor of one of the wards looking out on the gardener's quarters.

Following the resignation of Dr. Gannett, Dr. Henry F. Sears (donor of the Sears laboratory to the Harvard Medical School), who had been serving as assistant pathologist, was appointed his successor. Within the year Dr. Sears resigned and Dr. William T. Councilman, who had just been elected Shattuck Professor of Pathology at the Harvard Medical School, became pathologist and Dr. Mallory was appointed assistant to the pathologist. The primitive quarters assigned to the pathological department shocked Dr. Councilman, who came fresh from Johns Hopkins University, where pathology was given proper consideration. As the result of his efforts the trustees of the hospital prepared plans and the building occupied by the department up to now was constructed. Dr. Councilman served the hospital as pathologist up to 1911, when he resigned and was made consulting pathologist.

In 1892 Dr. William R. Stokes of Baltimore was appointed resident assistant pathologist. In 1895 Dr. James Homer Wright came from Baltimore to become second assistant to the pathologist. In 1896, when the Massachusetts General Hospital had constructed a building for the department at the behest of Dr. Councilman, Dr. Wright was appointed pathologist to that hospital. The writer became resident assistant pathologist in 1895, *vice* Dr. Stokes, resigned, and had the honor of serving in that capacity when the new building was opened in 1896.

The history of the pathological department, modernized and dignified by the efforts of Drs. Councilman and Mallory, is from this point largely a record of the work of Dr. Mallory.

Frank Burr Mallory came from Cleveland, Ohio, graduated from Harvard College with the degree of A.B. in 1886, and from the Harvard Medical School in 1890, with the degrees of A.M. and M.D. In 1891 he was appointed assistant to the pathologist at the Boston City Hospital and also assistant in histology at the Harvard Medical School. In 1892 he was made assistant in pathology at the medical school, meantime continuing as assistant to the pathologist at the hospital. The year 1893-94 was spent with Chiari in Prague and with Ziegler in Freiburg. On his return he resumed his former position at the hospital but was advanced to the position of instructor at the school. The hospital made him first assistant pathologist the following year and in 1896 he was appointed assistant professor at the school. In 1897 his title at the hospital was changed to assistant visiting pathologist and in 1900 to first assistant visiting pathologist. He was advanced to an associate professorship at the school in 1901, which position he held until 1919. In 1908 he became pathologist to the hospital and served in that capacity until his official retirement last year at the age of seventy. He was then made consulting pathologist. In 1928 he was appointed professor of pathology at the school and held this position until he retired.

During the years since his first appointment as assistant to the pathologist at the Boston City Hospital his activities have centered about that institution.

His publications, alone or with his colleagues or assistants, have covered a wide field. Beginning with a paper entitled "Sacro-Coccygeal Dimples, Sinuses and Cysts" during his first year as assistant in pathology, they include studies on typhoid, diphtheria,

pertussis, scarlet fever, measles, infectious lesions of blood vessels and the classification of nephritis.

His principal interests were reflected in a series of papers on improvements in histological technique beginning in 1895, which involved the working out of new staining methods that have carried his name to all quarters of the world. Particular attention was given to collagen (reticulum), neuroglia, myoglia and elastica, and a new element, the fibroglia fibril, was described in 1903. A collection of papers published from 1902 to 1920 dealt with the application of special histological methods to the study of tumors, with particular reference to the embryological origin of each type of cell entering into newgrowths, and the differentiation that each type undergoes. Mallory's studies in histological methods served to revolutionize the crude procedures in vogue, particularly in American laboratories, and gave a new impetus to the exact study and classification of tumors.

With a preliminary paper on necroses of the liver in 1901, and a second in 1911 on five types of lesions that may give rise to cirrhosis of the liver (which represented the carefully digested results of years of observation, and included demonstration of the hyaline change characteristic of alcoholic cirrhosis), Dr. Mallory's attention tended to focus itself more and more on diseases of the liver. When exact observation and the most careful histological technique could add little more to our knowledge of the cirrheses, resort was had to experimentation. These studies resulted in the demonstration that the lesions of pigment cirrhosis could be reproduced by the ingestion of copper, and the finding of copper in distilled liquors explained the relation that was known to exist between pigment cirrhosis and alcoholism. Hemochromatosis ceased to be a mystery. The alcoholic form of cirrhosis was a harder problem to solve. For over twenty years practically continuous efforts to produce this disease in a great variety of experimental animals have been carried on. Alcohol alone, the metals and any other agencies that suggested themselves, have been tried out without result. Recent work, however, indicates a successful end to this long search. Phosphorus, outstanding above all known substances, appears to show a highly selective poisoning effect on liver cells, even in infinitesimal doses, and a typical cirrhosis of alcoholic character has been produced in experimental animals.

One of the characteristics of Mallory publications, in addition to clearness of exposition, has been the insistence upon adequate illustration. From his first paper onward the quality of the drawings and photomicrographs has been the highest. Color was first used in a series of drawings illustrating a study of the staining reactions of neuroglia, in a paper in German in the *Centralblatt für allgemeine Pathologie und pathologische Anatomie* in 1895. In 1898 came the paper on the histology of typhoid, with its beautiful colored plates, which set a high standard for medical illustrations. In the more recent period, as he perfected and taught the technique of photomicrography, black and white reproductions, whose scientific exactness could not be questioned, tended to replace color. As a teacher and an editor he has insisted that high quality of illustrations is a prime essential for medical publications, and has helped to lift the level of American medical illustration to its present high point.

It is of interest that Dr. Mallory in 1906, in a paper, "The Present Needs of the Harvard Medical School," presented, not the needs of the laboratory branches, but the pressing want of endowment for clinical departments, with salaries large enough to permit clinical teachers to give up all of their time to the work.

Dr. Mallory, with Dr. J. Homer Wright, published first in 1897 "Pathological Technique," which became the laboratory bible for a generation, and embodied the principles laid down by Mallory with reference to clearness of exposition, conciseness and adequate illustration. In 1914 Dr. Mallory published "The Principles of Pathologic Histology."

In addition to the publications mentioned Dr. Mallory served from 1923 as editor-in-chief of the *Journal of Medical Research*. When the name of this journal was changed to *American Journal of Pathology* in 1925, he continued as editor-in-chief, in which position he is still as active as ever. He has also served as treasurer of the American Association of Pathologists and Bacteriologists since 1911. He is a member of the Commission on Standardization of Biological Stains, a corresponding member of the Royal Medical Society of Budapest, and an honorary member of the Philadelphia Pathological Society. He holds the honorary degree of Sc.D. from Tufts College, and a similar degree from Boston University.

More remarkable, however, than the production of publications has been the production of men. When the pathological department

of the Boston City Hospital was moved from its single room into the building now occupied, there was opportunity for expansion of the service, particularly in the direction of postgraduate teaching. Starting with a resident assistant pathologist and a single interne the staff has grown to include an assistant director, four assistants and five internes. Through this laboratory during the years has passed a notable group of young men, destined to attain distinction in the teaching of medicine. The stay in the laboratory has been limited, with few exceptions, to a period of three years or less. Each neophyte was required to progress through a series of assigned duties from the sharpening of knives, the practice of histological technique, bacteriological diagnosis, daily conferences on surgical diagnosis, autopsy technique with detailed reports on the gross and microscopic findings, to methods of teaching and of research. The work was laid out in such manner that the graduate from the department could step into a laboratory equipped to oversee every function of technician and diagnostician, and with a foundation that fitted him to carry out research, to teach the subject of pathology or apply its principles to the practice of medicine or surgery.

If he determined to enter one of the clinical medical branches as his life's work, he found himself prepared to visualize clearly the changes that the organs of his patients had undergone, and was enabled to apply more intelligently whatever therapy was indicated. If his chosen field were surgery, his training in surgical pathology made him competent to recognize gross pathological lesions as he met them, and adapt his operative procedures to the conditions discovered. If he entered the field of research, he was endowed with a knowledge of the reactions of the animal body that qualified him to understand and interpret the results obtained in his experimental researches.

Moreover his training was overseen personally by Dr. Mallory, with whom in frequent conferences his critical sense was ripened, he was taught the need of scientific accuracy, and a horror of slipshod methods. Constant association with an enthusiastic inquiring spirit served to inspire him with that scientific curiosity — that will to know — which is the *fons et origo* of medical progress.

Following is a list of the graduates of the department in chronological order.

- TIMOTHY LEARY. Professor Emeritus of Pathology, Tufts College Medical School. Medical Examiner, Suffolk County, Massachusetts.
- *RICHARD M. PEARCE. Formerly Professor of Pathology and Bacteriology, Albany Medical College, and Director, Bender Hygienic Laboratory, Albany, N. Y.; Professor of Pathology, University and Bellevue Hospital Medical College, and Director, Division of Medical Education, Rockefeller Foundation, New York, N. Y.
- LAWRENCE W. STRONG. Formerly Pathologist to the New York Eye and Ear Infirmary and the Woman's Hospital, New York, N. Y. Deer Isle, Maine, not in practice.
- JOSEPH H. PRATT. Professor of Medicine, Tufts College Medical School. Physician-in-Chief, New England Medical Center, Boston, Mass.
- * WILLIAM H. DOWNEY. Formerly in general practice, Peabody, Mass.
- WILLIAM H. BARTON. Medicine. Temple, Texas.
- D. HAROLD WALKER. Professor Emeritus of Otolaryngology, Harvard University Medical School. Visiting Otolaryngologist, Children's Hospital, Massachusetts General Hospital, and New England Hospital for Women and Children, Boston, Mass.
- GEORGE BURGESS MAGRATH. Professor of Legal Medicine, Harvard University Medical School. Medical Examiner, Suffolk County, Massachusetts.
- HARRY C. LOW. Visiting Surgeon, Little Wanderers Home, Boston, and Children's Sunlight Hospital, Egypt, Mass. Formerly Visiting Surgeon, Massachusetts General Hospital, Boston, Mass.
- FRANK T. FULTON. Consulting Physician and Cardiologist, Rhode Island Hospital, Providence. Consulting Physician, Providence City Hospital, Pawtucket Memorial Hospital, Woonsocket Hospital, and Westerly Hospital, Rhode Island.
- *H. A. STEENSLAND. Formerly Professor of Pathology, Syracuse University College of Medicine, Syracuse, N. Y.
- HENRY A. CHRISTIAN. Professor of Medicine, Harvard University Medical School. Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Mass.
- *WALTER R. BRINKERHOFF. Formerly Assistant in Pathology, Harvard University Medical School; Fellow of the Rockefeller Institute for Medical Research, New York, N. Y.; Physician to the Carnegie archeological expedition to the Transcaspian Territories; Director of the Molokai Leprosy Investigation Station of the United States Public Health Service, Hawaiian Islands; Assistant Professor of Pathology, Harvard University Medical School, Boston, Mass.
- *ELMER E. SOUTHARD. Formerly Professor of Neuropathology, Harvard University Medical School, and Director of the Boston Psychopathic Hospital, Boston, Mass.
- RALPH L. THOMPSON. Professor Emeritus of Pathology, St. Louis University School of Medicine. Director, National Pathological Laboratory, St. Louis, Mo.
- RALPH E. LEE. Douglaston, N. Y., not in practice.
- NATHANIEL H. GIFFORD. Surgery. Providence, R. I.

* Deceased.

- S. BURT WOLBACH. Professor of Pathology, Harvard University Medical School. Pathologist, Peter Bent Brigham Hospital, Children's Hospital, and Boston Lying-in Hospital, Boston, Mass.
- CHARLES W. DUVAL. Professor of Pathology, Tulane University of Louisiana School of Medicine, New Orleans, La.
- *PAUL A. LEWIS. Formerly Assistant Professor of Pathology, University of Pennsylvania School of Medicine; Director, Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.; and Associate Member, Department of Animal Pathology, Rockefeller Institute, Princeton, N. J.
- GEORGE S. GRAHAM. Professor of Pathology, Graduate School of Medicine of the University of Alabama, Birmingham, Ala.
- SAMUEL T. ORTON. Professor of Neurology and Neuropathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.
- HAROLD E. ROBERTSON. Professor of Pathology, University of Minnesota Medical School, Minneapolis. Pathologist, The Mayo Clinic, Rochester, Minn.
- *OLIVER R. MABEE. Formerly in the practice of surgery, Toronto, Canada.
- BURDETT L. ARMS. Farmington, Maine. Formerly Bacteriologist, Division of Diagnostic Laboratories, State Board of Health, Jacksonville, Fla.
- LAWRENCE J. RHEA. Associate Professor of Pathology, Faculty of Medicine, McGill University. Pathologist, Montreal General Hospital, Montreal, Canada.
- ARTHUR B. EMMONS. Executive Director, American National Red Cross, Boston Metropolitan Chapter, Boston, Mass.
- *FREEMAN D. BOSWORTH. Formerly in practice, Stockbridge, Mass.
- SAMUEL R. HAYTHORN. Professor of Preventive Medicine, University of Pittsburgh School of Medicine. Director, William H. Singer Memorial Research Laboratory, Pittsburgh, Pa.
- THOMAS ORDWAY. Professor of Medicine and Dean, Albany Medical College, Albany, N. Y.
- ARCHIBALD W. HUNTER. Visiting Surgeon, Vancouver General Hospital. Pathologist to the Coroner, City of Vancouver, Vancouver, B. C.
- HARRY S. BERNTON. Associate Professor of Hygiene and Preventive Medicine, Georgetown University School of Medicine, Washington, D. C.
- OLIVER S. HILLMAN. Clinical pathology. Instructor, New York Post-Graduate Medical School and Hospital, New York, N. Y.
- DANIEL J. HURLEY. Surgical Examiner, U. S. Veterans Bureau, Boston. Member, Massachusetts Board of Registration in Medicine, Boston, Mass.
- ALEXANDER M. BURGESS. Assistant Professor of Biology, Brown University. Physician-in-Chief, Department of Tuberculosis, and Visiting Pathologist, Providence City Hospital, Providence, R. I.
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- FRANCIS F. HENDERSON. Visiting Surgeon, Boston City Hospital, New England Sanitarium and Hospital, and Charlesgate Hospital, Boston, Mass.
- STUART GRAVES. Professor of Pathology and Dean, University of Alabama School of Medicine, University, Ala.
- FRANK A. MCJUNKIN. Professor of Pathology, Loyola University School of Medicine, Chicago, Ill.
- L. WHITTINGTON GORHAM. Professor of Medicine, Albany Medical College, Albany, N. Y.
- EDGAR M. MEDLAR. Director, Hegeman Memorial Research Laboratory, Mount McGregor, N. Y.
- LLOYD P. MACHAFFIE. Medicine. Ottawa, Canada.
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- PAUL R. HINCHEY. Surgical Intern, Boston City Hospital, Boston, Mass.
- WILMER C. SMITH. Surgical Intern, Boston City Hospital, Boston, Mass.

In addition to this group of graduates a number of men, including scholars of the National Research Council and the Rockefeller Foundation, have spent varying lengths of time in the laboratory.

As one looks back over the record of these productive years one is inclined to believe that the remarkable results that have been obtained represent more than the activities of a single man. They have more the character of the products of a vital force. And it is a pleasure to report that this vital force, which is Frank Burr Mallory, is still as active, alert and delightfully enthusiastic as it has been for forty odd years. Moreover, the production of men is still being

carried on under Dr. Mallory's kindly oversight as a volunteer, by the man he trained and selected to be his successor, Dr. Frederic Parker, Jr.

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Since the medical examiner service of Suffolk County, Southern district, has been housed with the pathological department of the hospital for years, and its association with the department has become very close, a word about its origin and development is perhaps desirable.

The medicolegal relation of the hospital receives its first notice in the annual report of 1871, with a reference to the establishment of facilities for the reception and identification of the unknown dead found elsewhere.

Up to 1877 Massachusetts had a coroner system inherited, through the colonial and provincial laws, from the Anglo-Saxon practice. Scandals that arose in Suffolk County, which includes Boston, led to the abolishment of the coroner and the replacement of this anachronistic system by the medical examiner system. This change was brought about by the combined action of the Massachusetts Bar Association and the Massachusetts Medical Society. The coroner was called upon to exercise both medical and legal functions with requirements beyond the limitations of most human capacities. Developed in the early years of Anglo-Saxon civilization, when the coroner actually represented the crown in legal affairs, the clumsy inefficiency of the system became manifest as judicial and police systems were developed. When a man dies as the result of violence two basic questions require an answer. The first, "What caused the death?" calls for the exercise of purely medical functions. The second, "Who caused the death?" is a matter for the police and the courts. The new law placed the responsibility of determining the cause and manner of the death upon medical examiners, men presumably selected for their fitness to answer the requirements. The determination of who might be the guilty party, and the degree of his guilt, was left to the police and the courts.

Two medical examiners for Suffolk County were appointed to replace the forty-three coroners who were in office when the law was passed. The county was divided by agreement, the Southern district focussing at the Boston City Hospital. Dr. Frank W. Draper,

who was connected with the staff of the hospital, was appointed medical examiner and served until 1905. Dr. William McDonald, who replaced him, died in 1910. The writer, who had been associate medical examiner since 1908, was then appointed medical examiner and has served since. In 1913 the addition of a second floor to the wing of the pathological building furnished quarters for the medical examiner service.

The kindly coöperation which Dr. Mallory has practised and the close association with his progressive department have helped to bring about whatever success has been achieved by the medical examiner service in this district.

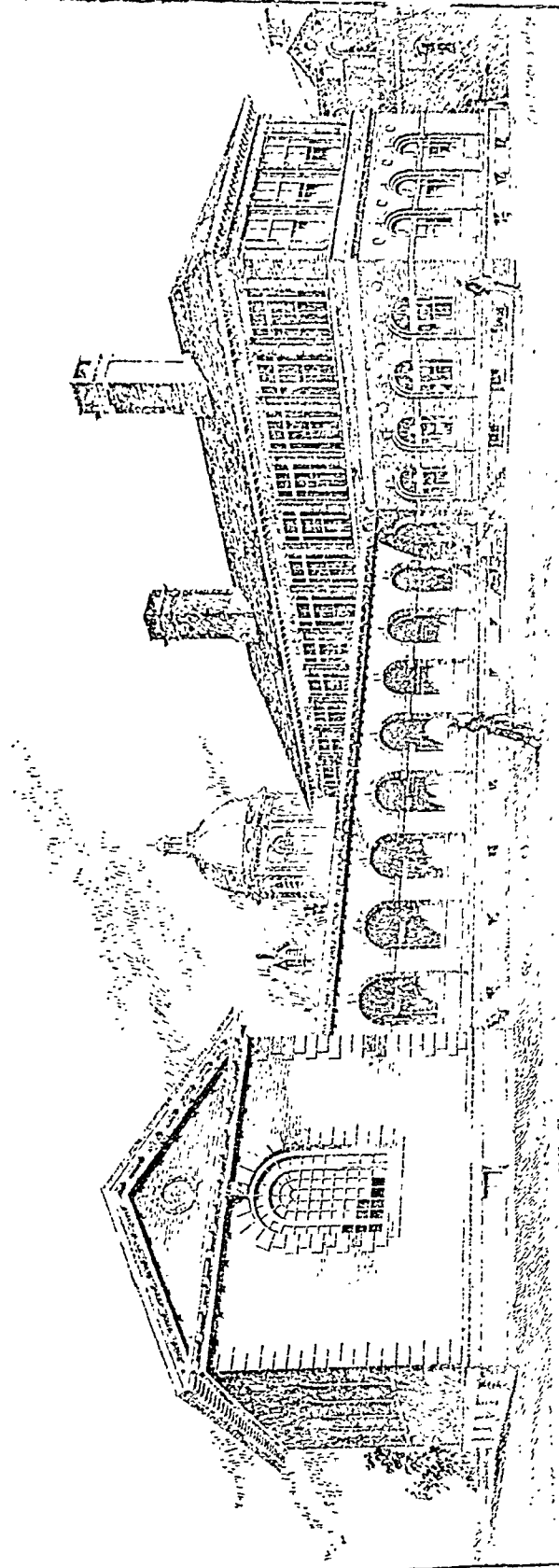
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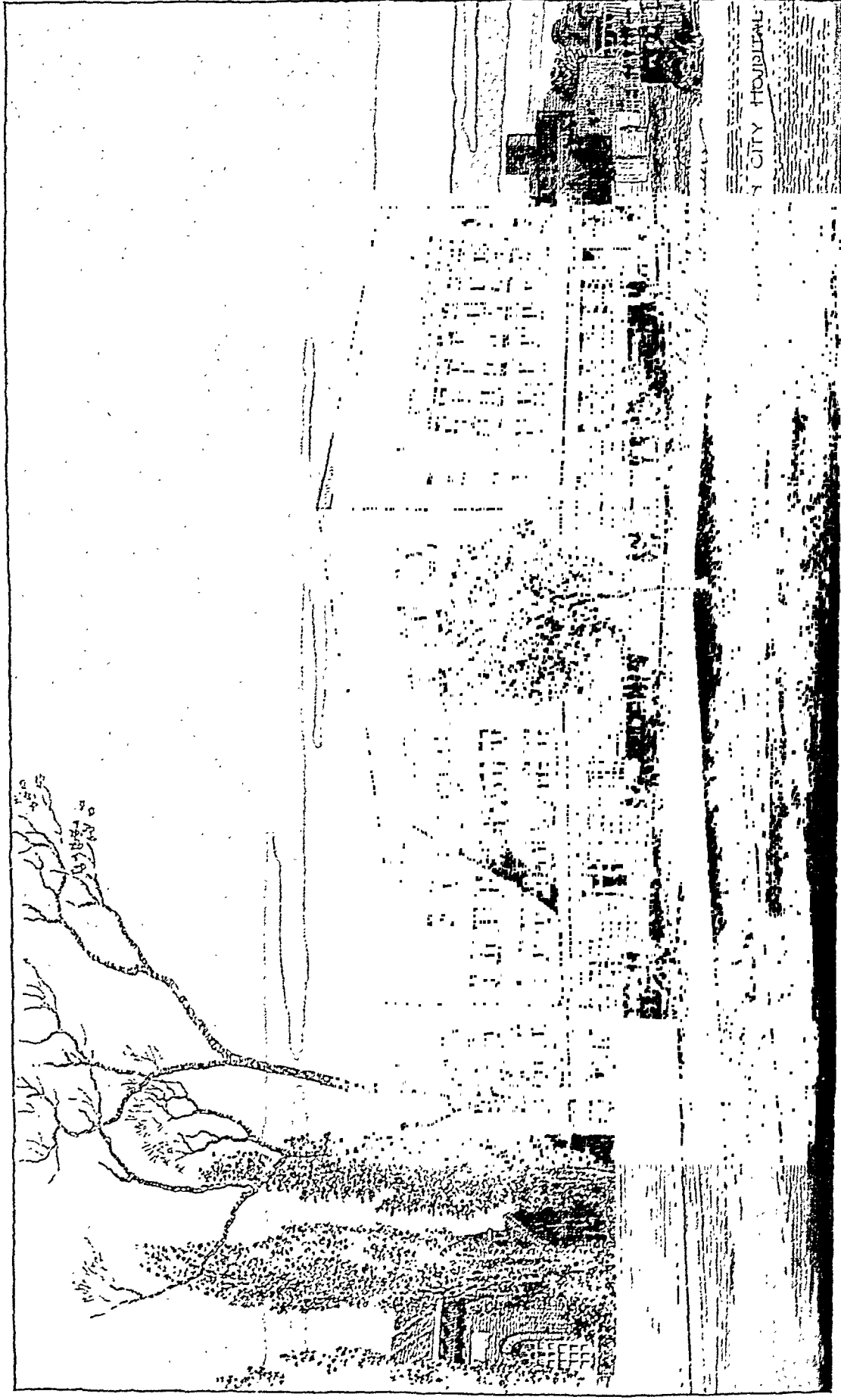
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It is fitting that the new building which is to harbor the department that has grown so great under his hands should bear the name of Mallory. The building houses more than the department of pathology, since it will include offices and facilities for the medical examiner service. It is properly named "Mallory Institute of Pathology."

BOSTON CITY HOSPITAL
PATHOLOGICAL BLDG. AND MORTUARY CHAPEL
EDWARD M. WHEELWRIGHT, CITY ARCHITECT



THE ORIGINAL PATHOLOGICAL BUILDING



THE MALLORY INSTITUTE OF PATHOLOGY OF THE BOSTON CITY HOSPITAL

THE MALLORY INSTITUTE OF PATHOLOGY *

ROBERT N. NYE, M.D.

(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

The old Pathological Laboratory of the Boston City Hospital was formally opened in December, 1895. That the building of this unit was thought by the Trustees to involve a considerable, but fully justified, expense can be gained from their thirty-second annual report, published in 1896: "No modern hospital . . . can be considered as having the proper facilities for doing scientific medical work without a well-equipped laboratory. The Trustees believe that, while the cost of these laboratories has been large, they have exercised wisdom in affording to the professional staff of the hospital every facility for the treatment of patients fully within the most advanced lines of modern scientific medicine." During the next few years the capacity of the hospital was rated at about 800 beds. The staff of the laboratory consisted of a pathologist, two assistant pathologists, two assistants, two pathological internes, four clerical and technical assistants, and in addition the personnel of the medical examiner's office for Suffolk County, South. The routine work of the laboratory averaged, annually, 230 autopsies, 750 surgical specimens, 1100 bacteriological examinations and about 18,000 throat cultures for diphtheria bacilli; the annual expense was about \$9000. Teaching was confined to students from Harvard Medical School. Medicolegal cases averaged about 500 a year.

Due to the greatly increased demands placed on the Boston City Hospital and to the obsolescence of many of the buildings, an extensive building program was started about ten years ago. Many of the oldest buildings have been replaced, while others have been modernized. The Trustees plan, eventually, to increase the capacity of the Main Department to well over 2000 beds; at present it is about 1450 beds. These, together with 300 beds at the South Department and 625 beds at the Sanatorium Division at Mattapan, give a total capacity of 2375 beds. The work of the laboratory has increased proportionately. Last year about 700 autopsies were per-

* Received for publication May 15, 1933.

formed and, at the present rate, there will be nearly 800 this year. Approximately 4300 surgical specimens are handled and about 8500 bacteriological examinations made, in addition to 23,000 diphtheria release cultures and 700 pneumococcus typings. About 22,000 Kahn tests are performed annually. Over 1300 cases are certified by the medical examiner or his assistant each year. The laboratory staff consists of a consulting pathologist, one pathologist, two assistant pathologists, three assistants, five pathological residents, seven technicians and one stenographer, and in addition the medical examiner, two assistant medical examiners, one technician and two stenographers. The maintenance of the laboratory proper requires an annual expenditure of about \$50,000. Positions in the departments of pathology of Harvard, Tufts, and Boston University Medical Schools are held by members of the staff and a considerable amount of their time is devoted to routine pathological teaching, pathological conferences and postgraduate teaching.

In view of the marked increase in activities it is not surprising that the Trustees, once again, felt obliged to provide adequate laboratory facilities and a new building was included in the expansion program. Land on the easterly corner of Massachusetts Avenue and Albany Street, just across from the Main Department, was purchased in 1931, and in 1932 a sum of \$670,000 was appropriated for the building. All the bids on the general contract were surprisingly low and the building was completed at a cost of approximately \$460,000, including extras and architects' fees. Ground was broken in August, 1932, and the building was ready for occupancy just about a year later.

The Trustees were most generous in allowing the senior members of the laboratory staff unlimited freedom in planning the interior arrangement and in specifying the fixed equipment; and the architects, James H. Ritchie and Associates, did their utmost to conform to these plans and specifications. The sum of \$80,000 was appropriated for furnishing and equipping.

GENERAL PLAN OF BUILDING

The building has a frontage of 111 feet on Massachusetts Avenue and extends back 183 feet. It consists of a basement, four main floors and a penthouse. Above the first floor the building is U-shaped, with

the open end toward Albany Street; the base is 183 feet long and each wing 62 feet long, all being about 50 feet in width. The steel framework rests on deep concrete caissons. The floor and roof slabs are of concrete, conforming to "steel tile" construction, and in general, all partitions consist of gypsum or terra-cotta blocks. The volume of the building is slightly over 1,000,000 cubic feet.

The exterior finish is red brick with cast stone trim. The front is moderately decorated with bas reliefs in cast stone. Between the pilasters and the windows of the second and third floors are seven cast aluminum panels with a design in bas relief, containing, as a central motif, the bishop's cap and rose of the Barber Surgeons of London. These panels are repeated on the ends of the wings, and the spandrels above the third floor windows are ornamental discs containing, as motifs of health, medicine and surgery, respectively, the radiant head of Apollo from the Physicians of London, the caduceus and the medieval lancets from the Barber Surgeons of London.

There are 361 windows, exclusive of the garage. These are located, chiefly, on the second and third floors. Practically all of the animal rooms on the fourth floor are lighted from above by skylights. Electrically and manually operated opaque shades are provided, respectively, in the amphitheater and in the photographic room and museum on the second floor and the conference room. The lower sashes of the larger windows in the autopsy rooms and both sashes of the standard windows in the autopsy rooms, undertakers' rooms and toilets are glazed with Seynite glass.

The floors of the corridors are covered with green asphalt tile, except in the basement and on the fourth floor, where terrazzo or a granolithic finish is used. All laboratory floors are covered with green linoleum and those of the entrance lobby, amphitheater, library and offices with rubber tile. In all toilets, storerooms, autopsy rooms, animal rooms and others that are apt to be wet, terrazzo or granolithic floors are used. The walls, in general, are of plaster on terra-cotta or gypsum blocks and are painted a pale green. In all storerooms and animal rooms, buff paint has been applied directly to the smooth faced terra-cotta blocks. The walls of the mortuaries, undertakers' rooms and small autopsy room on the fourth floor consist of white Ar-ke-tex tile. The ceilings, as a rule, are the painted undersurfaces of the floor slabs. In some instances they are furred down and plastered to cover the service piping, thus improving the

attractiveness of the room, as in the library, offices and entrance lobby. Otherwise, all piping is exposed.

Natural ventilation is used in the majority of the laboratories. In the amphitheater, large autopsy rooms and large conference room, heated air is supplied and exhausted. In the two stock animal wings heated air is supplied in the corridors and drawn through ventilating panels in the doors by exhausts located in each room. In certain other rooms, such as all toilets, the two darkrooms, the undertakers' rooms and the mortuary rooms, air exhausts only are provided. Large hoods connected with power exhaust fans are placed over all sterilizers and gas ranges. All chemical hoods are provided with individual exhaust fans.

The electric lighting fixtures are in part of a type that controls the areas of light distribution. The autopsy tables are lighted by three or five Holophane ceiling boxes, each containing a 150 watt lamp, reflector and lens. This results in an even, shadowless illumination, equivalent photometrically to 100 to 150 foot candles.

The vacuum system of steam heating is used and is controlled in each room automatically. In all of the laboratories with fixed counters or benches the radiators are placed beneath the windows with outlet grills set into narrow shelves at the level of the window ledgers. Celotex fronts on the sheet metal radiator cabinets protect against excessive radiation underneath the counters. As mentioned previously, the amphitheater, two large autopsy rooms and a conference room are heated indirectly. The stock animal wings are heated to about 50° F. indirectly, but each room can be raised to a higher temperature by means of auxiliary radiators, automatically controlled.

Electricity, gas and compressed air are supplied to all the laboratories. At least one complete set of outlets on the counter and one on a small service shelf on the side or back wall are provided in each room. Both the lighting and power lines are 110 volts D.C., but the autopsy rooms and the physiological laboratory are also supplied with 110 volts A.C. for use with a portable roentgen-ray machine. Medium pressure lines furnish steam at 60 pounds for all the chemical hoods, sterilizers and stills. There is an automatic water still and storage tank on the fourth floor and the latter is connected to outlets in the corridors of the second and third floors by means of block tin-lined brass pipe.

There are two elevators — a large service elevator, manually controlled, and a small automatic passenger elevator. A dumb-waiter for gross specimens and heavy glassware extends from the basement to the fourth floor. A rubbish chute goes from the third floor to the basement. All rubbish and refuse on the fourth floor is disposed of in a gas-fired incinerator.

Refrigerating machinery in the basement furnishes brine for the mortuaries, cold rooms and a cooler for the water to the drinking fountains. An electrically heated incubator room is built into the bacteriological suite on the second floor.

Both the fixed and movable equipment have been standardized as much as possible. The counters, or benches, are 30 inches high, 24 inches deep and are set against 6 inch aprons, dropping down from service shelves 12 inches wide, at the level of the window ledges. On the face of this apron are placed the various service outlets, and the heat outlet grills are set flush with the top surfaces of the service shelves. The tops of the counters are made of 4 inch birch strips $1\frac{3}{4}$ inches thick, and have the common black acid-proof finish. Drawers and cupboards, underneath, are placed so that they do not interfere with space in front of windows. An overhead bench light duplicates each window unit.

The majority of sinks are built of alberene stone with drain boards of the same material. They vary considerably in dimensions according to the location. Each is provided, in general, with a lighting fixture, needle valve washing outlets, a removable peg board, and a substantial overhead shelf, on the underside of which are adjustable grooves for holding graduated cylinders.

Nearly all of these cupboards, cabinets and cases are movable. They are constructed of wood and have a unit width of 4 feet. Those with an upper section have a usable height of 7 feet. Tables are 5 or 10 feet long and 30 or 36 inches high; the tops are usually of wood with acid-proof finish, but some are of rubber or of alberene stone.

All the storerooms are equipped with metal shelving of dimensions adapted to the particular use of each room.

BASEMENT

A passenger and service tunnel from the Main Department enters the building in the northeast corner of the basement.

The area under the ambulance garage is unexcavated. A large storage room for autopsy specimens and general supplies occupies practically all of the space under the north wing, and three rooms in the immediate vicinity are required for the refrigeration machinery, main electric switchboard and the air compressors and vacuum pumps of the heating system.

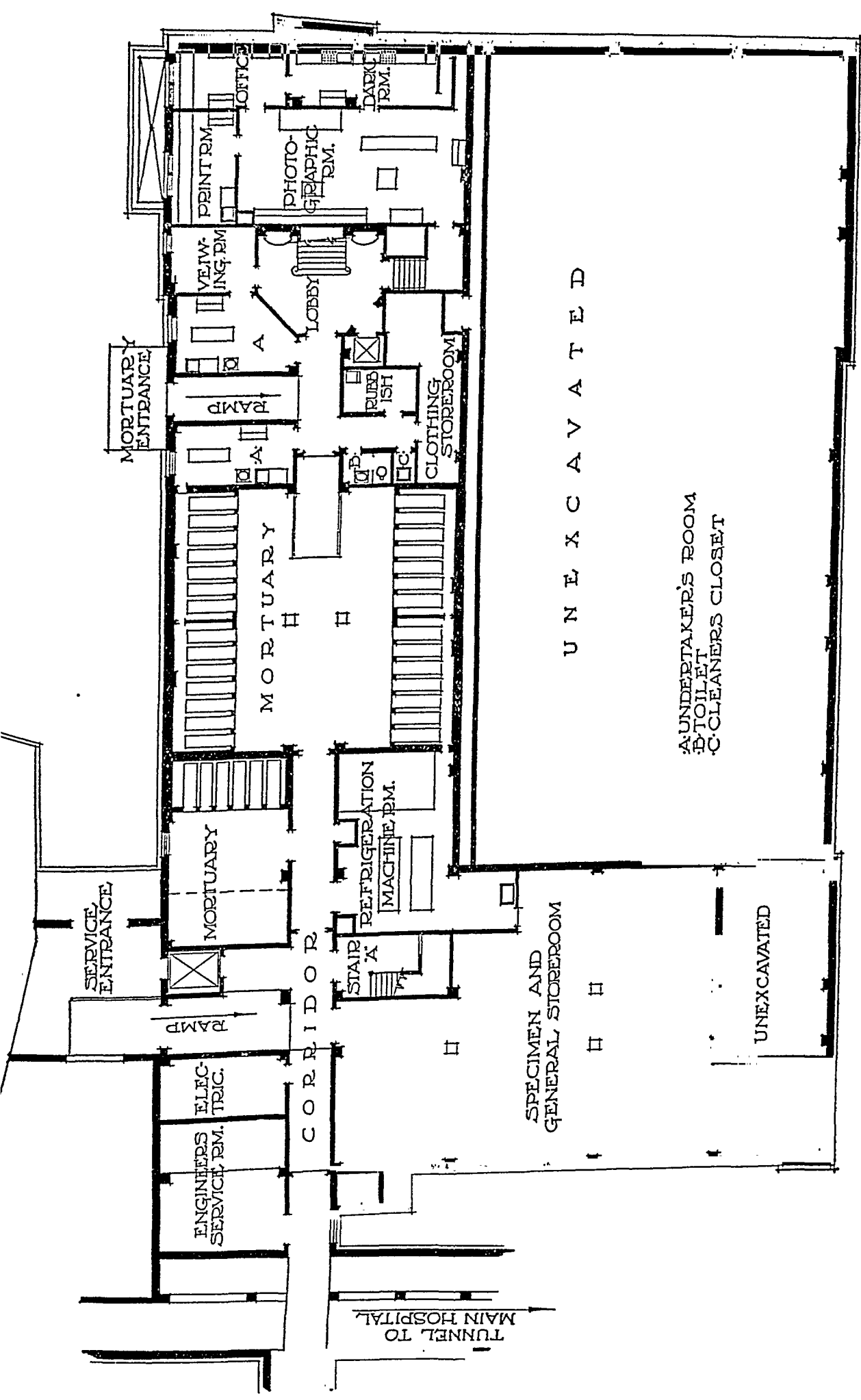
The main mortuary is centrally located, with one entrance from the corridor connecting with the tunnel and another connecting with an outside loading platform on the east side of the building. There are 2 refrigerated compartments, each containing 42 trays and cooled to about 34° F. There is an auxiliary mortuary with 1 box and 17 trays, with provision for an additional box of equal size. This is cooled to about 25° F. and is used for unclaimed bodies and for the unpleasantly odoriferous ones of certain medicolegal cases. At each side of the corridor to the loading platform is a room equipped with a standard embalming table, sink, lavatory and cabinet for the convenience of the undertakers. From one leads a small viewing room, which, in turn, opens into the hall at the foot of a short flight of stairs from the entrance lobby.

The photographic unit occupies the end of the basement and the floor grade is 4 feet lower than the level of the main basement. There is a large inside room completely equipped for photographing charts, and gross and microscopic specimens, for enlarging and reducing, for projecting lantern slides, and for the storage of negatives. A large darkroom with a special sink and a printing booth connects with the inside room by door and by maze and with a small office by another door. The office and a print storage room both receive daylight from an areaway.

FIRST FLOOR

All the space beneath the south wing and that between the two wings is occupied by an ambulance station. There is no connection between this garage and the rest of the building.

An amphitheater occupies practically all of the north wing, extending up to include the second floor. Entrance is available from



TUNNEL TO
MAIN HOSPITAL

ENGINEERS ELEC.
SERVICE RM.

RAMP

SERVICE
ENTRANCE

MORTUARY

MORTUARY

MORTUARY
ENTRANCE

VIEW-
ING RM.

PRINT RM.

PHOTO-
GRAPHIC
RM.

DARK
RM.

LOBBY

DARK

REFRIGERATION
MACHINE RM.

STAIR

CLOTHING
STOREROOM

PURB
ISH

SPECIMEN AND
GENERAL STOREROOM

UNEXCAVATED

U N E X C A V A T E D

A-UNDERTAKER'S ROOM
B-TOILET
C-CLEANERS CLOSET

the first or second floors of the main building or from a doorway on Albany Street, which connects by a stairway to the back of the amphitheater on the second floor. The amphitheater has a seating capacity of 148. The demonstration bench is arranged with a hidden sink and service outlets, so that the room can be used as a chapel, when necessary. On the front wall are three sections of blackboard and, above, two chart racks. The wall space between the latter serves as a projection screen and the level of the floor of the projection room is such that the midpoint of the horizontal projecting ray is centered in this space. The projection room is built to conform to the requirements of the building code for motion-picture projection. The opaque window shades are controlled by a switch on the front wall, and a buzzer in the projection room is operated by a cord button, similarly located. The amphitheater preparation room is furnished with a sink, lavatory and cabinets, and is of sufficient size for the storage of charts and microprojection apparatus.

Four rooms are used for storage purposes. One for paraffin blocks has brine coils automatically controlled so that the room temperature never goes above 65° F., even in the hottest weather. Two others are for surgical specimens and a fourth for jars and other heavy glassware. There is a washroom connecting with the latter.

The museum is a room 61 feet long and 19 feet wide and is without windows. The specimens are kept in dust-proof steel museum cases and are adequately lighted by special fixtures. On the opposite side of the hall are an office for the curator and a museum preparation room which, in addition to the standard laboratory equipment, contains a large motor-driven band saw.

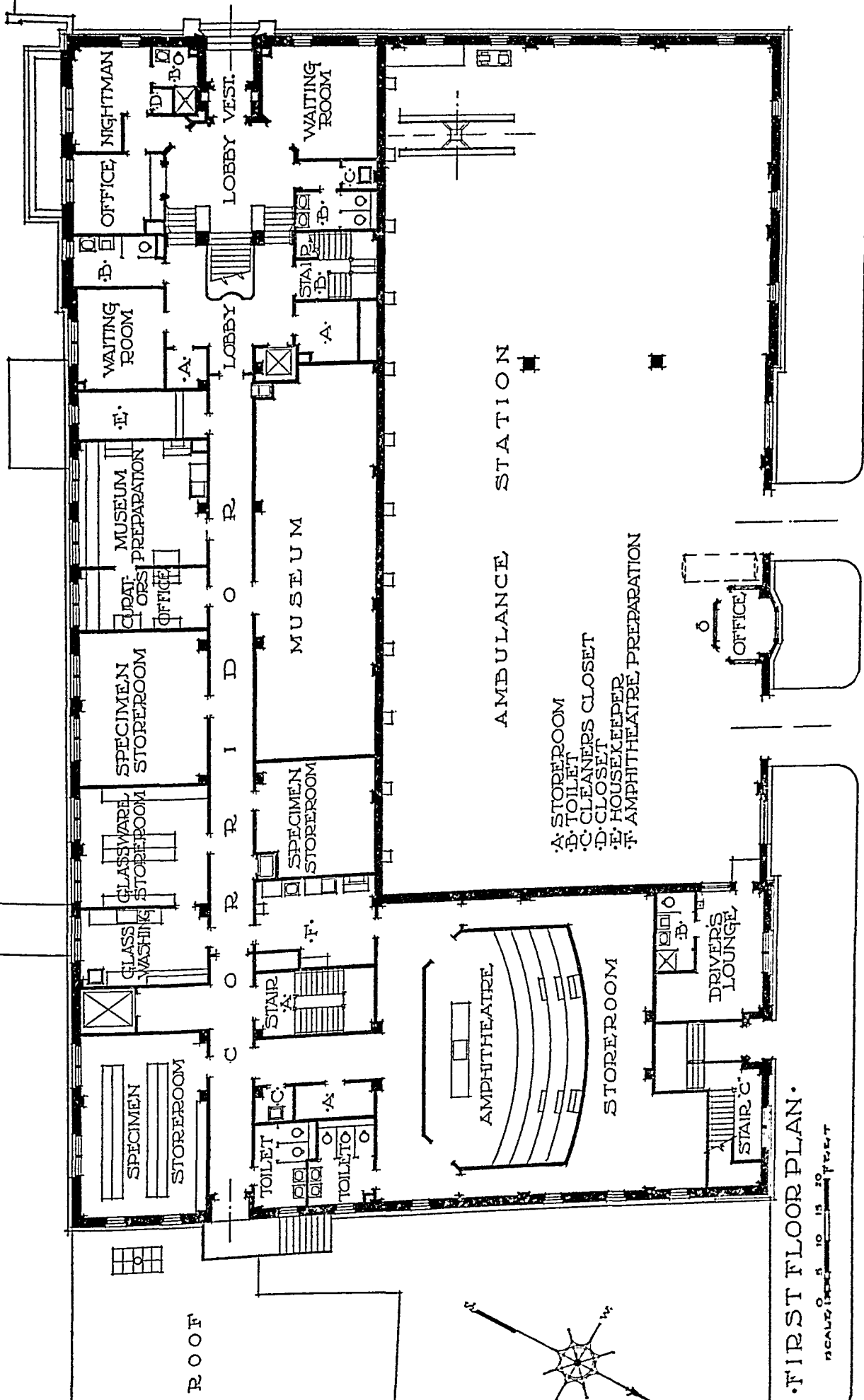
The main entrance lobby is at a lower floor level than the remainder of the first floor. Opening from this are a waiting room, an office, and a bedroom and bath for the man on night duty. At the higher level, but still a part of the same unit, are an additional waiting room, a housekeeper's room, small storerooms and toilet facilities.

SECOND FLOOR

The north end of the main part of the building is devoted to bacteriology and serology. The suite for routine bacteriology consists of a utility room and a bacteriological laboratory connected by a narrow passageway. On the outer side of this passageway are two

SERVICE YARD

ROOF



FIRST FLOOR PLAN.

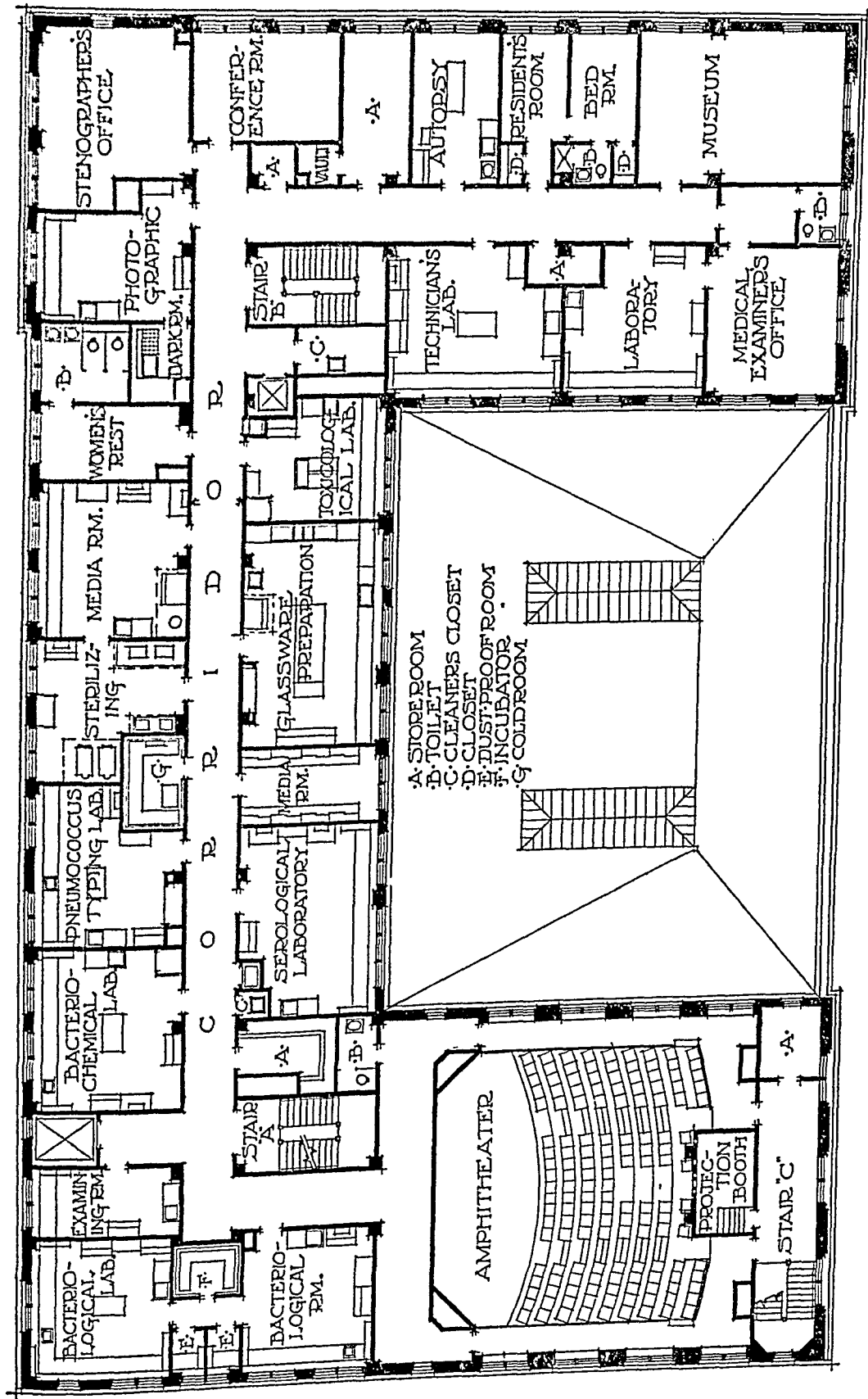
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"transfer" rooms. These two rooms have air-tight doors and are ventilated by fans with automatic shutters, set into the upper sashes of the windows, air being drawn in through air filters. On the opposite side of the passageway is an electrically heated incubator room with suitable controls for maintaining an even temperature of 37.5° C. There is a small room which is used, chiefly, for examining patients and a larger one equipped with a chemical hood. A special room is provided for pneumococcus typing. It contains a built-in ventilated cabinet for holding mice jars. All the bacteriological rooms have small staining sinks built into the counters. The serological room contains the usual laboratory equipment, together with a generous supply of outlets to ensure adequate connections for various water baths.

Three rooms may be grouped as a sterilizing and glassware and media preparation unit. One room contains two autoclaves and hot-air ovens, an Arnold sterilizer and an inspissator. This connects directly with the media room, which, in addition to standard equipment, is provided with a 10 gallon steam-jacketed tilting kettle of Allegheny metal and a 6 burner gas range. Across the hall is a large room for the washing and preparation of glassware. There is a special sink with duplex washing and rinsing compartments, a small sink directly in front of one set of windows, a hopper, a large gas range and a steam-heated drying cabinet. The counter and table tops are covered with rubber to minimize breakage.

Set between the bacteriological group and this last group are two rooms for common use. The walls of one are lined with built-in cabinets for the storage of sterile glassware and media. The other is a cold room kept automatically at about 35° F. The latter contains a special freezing compartment cooled to about 20° F. and a standard counter with the usual service outlets.

The remainder of the second floor is devoted to the activities of the medical examiner. In addition to a private office and laboratory ample room is provided for stenographers, files and storage. There are a small chemical laboratory, a technicians' room, a photographic room with an adjoining darkroom, a private autopsy room, a museum and a women's restroom.



SECOND FLOOR PLAN.

SCALE 1" = 5' 10" 20' 7" 1/2"

THIRD FLOOR

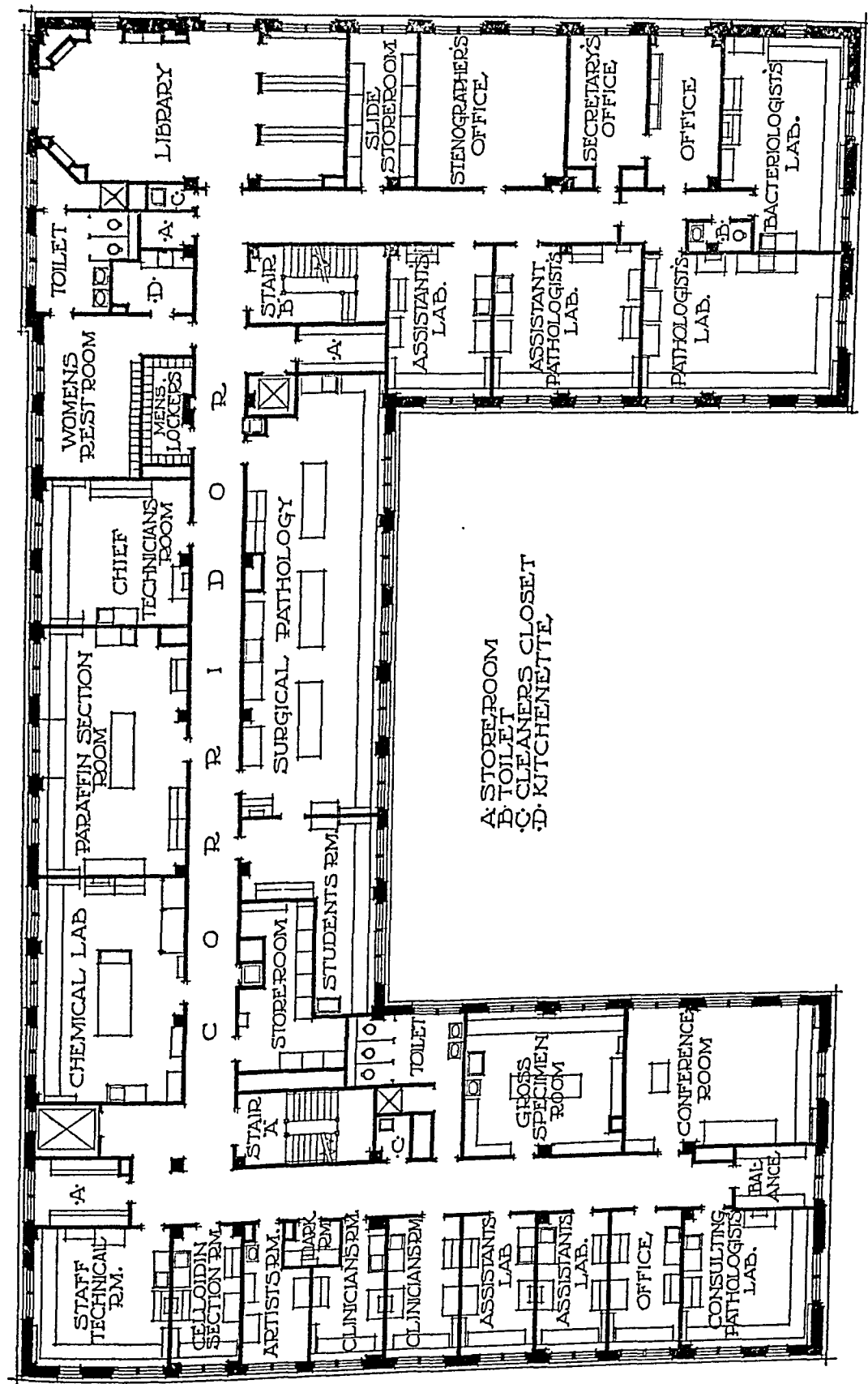
The entire third floor is assigned to the routine pathological work of the hospital.

In the extreme end of the north wing is a conference room with a seating capacity of about 30. This room is equipped with manually operated opaque shades and a screen for microprojection. At the front of the room are blackboards and the rear counter has an abundance of electrical outlets for microscopic demonstrations. Next to this room is a room for the storage of gross specimens. Pipe frame racks with alberene shelves are set against the walls for the agate-ware tanks. The counter under the windows and the large demonstration table in the middle of the room have alberene tops. The floor is of terrazzo and has a drain.

In addition to a balance room at the end of the corridor, the remainder of the wing is occupied by two large and seven small laboratories and a darkroom. The two larger rooms are used by the consulting pathologist and for a staff technical room, respectively. The smaller rooms are occupied by assistants and clinicians, with one devoted to the cutting of celloidin sections.

The north side of the main part of the building furnishes ideal natural light for microscopic work and the space is utilized for a large room for surgical pathology and a smaller one for special and graduate students, with generous counter space for 10 and 5 men, respectively. In the main room, at one end, are provisions for examining and preparing surgical specimens. The bottles of the celloidin series are kept on tables in the center of the room and there is a special sink with 20 washing outlets for use following Zenker fixation. Across the hall are a completely equipped chemical laboratory, a room for cutting paraffin sections, a room for the chief technician, a men's locker-room and a women's restroom with kitchenette attached.

The south wing is consigned to the staff and records. In connection with a secretary's room and office are the laboratories of the pathologist and bacteriologist. Two laboratories for the assistant pathologist and first assistant are provided. There is a library with a small stackroom, and next to the stenographers' and record room is one for slide storage cabinets.



•THIRD FLOOR PLAN•

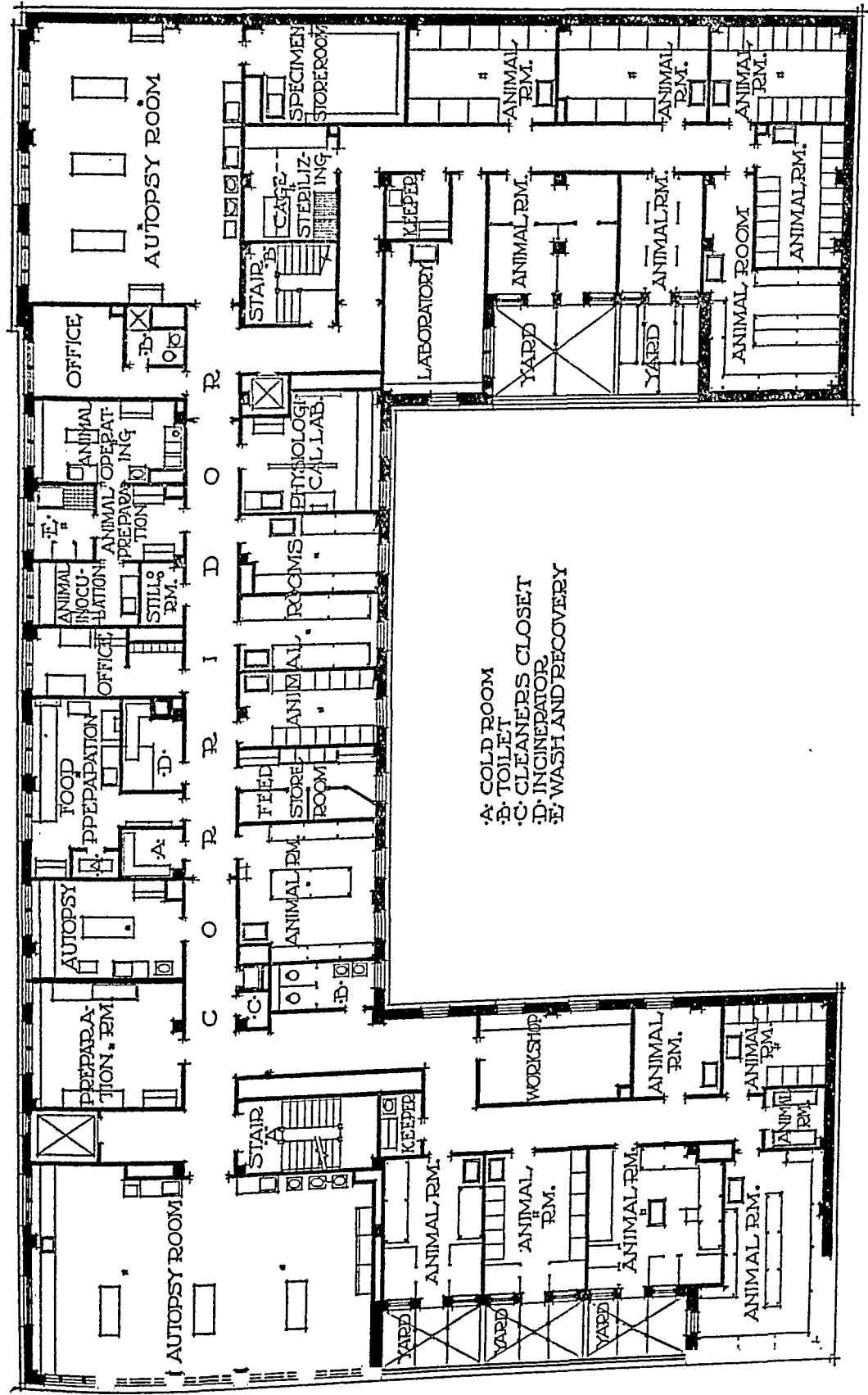
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FOURTH FLOOR

Practically all of the north and south wings are used for stock animal rooms and outside pens. Each wing has a small room for the man in charge. The equipment of the individual rooms depends upon their purpose. Each is provided with a sink, sill cock and floor drain. Pressure steam is piped to each corridor and a hose of sufficient length to reach the far end of the farthest room is provided. Fixed sheet metal cages with mastic floors and set on concrete bases are furnished, in general, for rabbits, guinea pigs, dogs and cats. There are larger grilled pens for the dogs and grilled cages for monkeys and birds. Angle-iron racks suspended from the ceiling are furnished to hold individual cages for mice, rats, monkeys, birds and male rabbits. There are two inside and two outside pens for sheep. These wings also contain a workshop and a room for cage cleaning and sterilizing.

The main routine and medicolegal autopsy rooms are located at the north and south ends, respectively, of the main building. They have a higher ceiling (12 feet) than the other rooms and are lighted, chiefly, by large windows closely placed and running up to the ceiling. The floors are of a grayish green terrazzo with a greenish black border and base. The walls, above a 5 foot dado of light green matt glazed tile, topped by a narrow band of black, are white, as are the ceilings. Large white enamel sinks, lavatories and instrument sterilizers are fixed against the walls. The autopsy tables are built of stainless steel and the movable furniture, such as instrument cabinets, observation stands and tables, is finished in white enamel. Standard sets of service outlets are available on the walls near the autopsy tables and in the routine autopsy room a standard counter runs along one side of the room. The latter contains a staining sink and is provided with equipment for frozen sections. There is a small autopsy room near the main room, which is used for cases of contagious disease and for private autopsies. Between the two is a preparation room. All Zenker and gross fixation on autopsy material is completed in the latter. In connection with the medicolegal autopsy room are a small office and a room for storing gross specimens.

The central part of the main building houses a unit concerned, chiefly, with work on experimental animals. From an inner hall, used for animal preparation, open an inoculation room, an animal



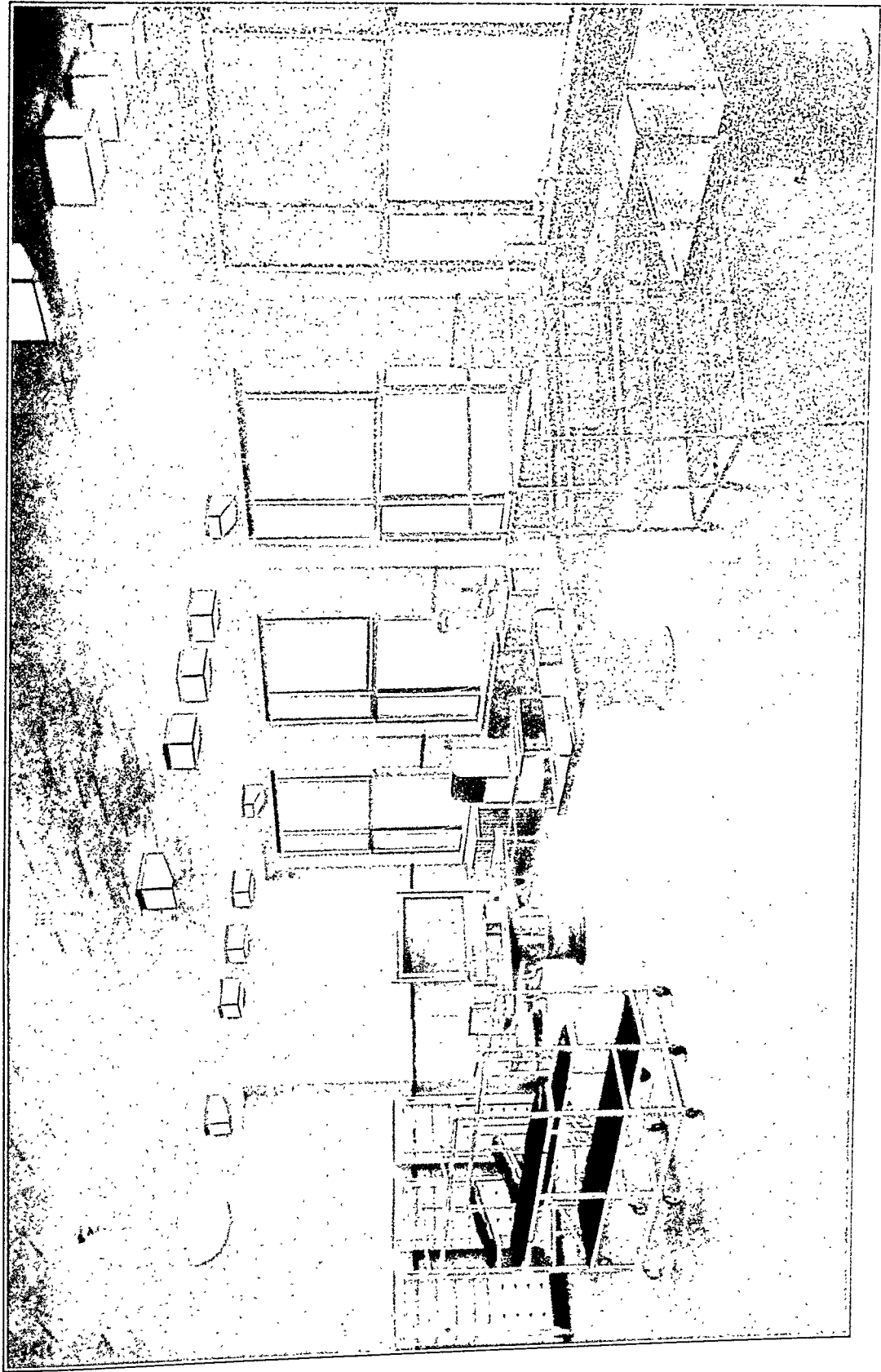
FOURTH FLOOR PLAN.

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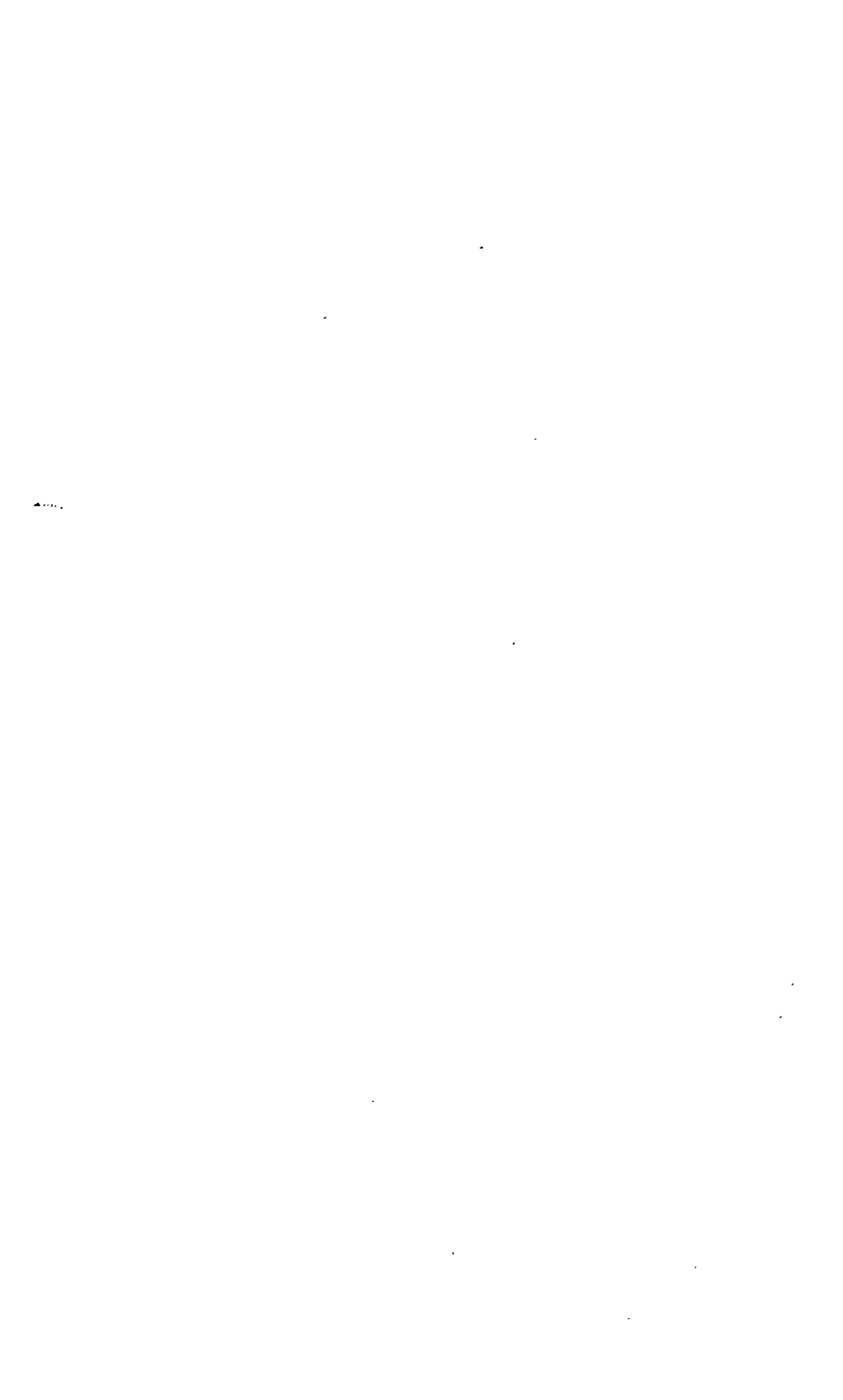
operating room completely equipped, and a small room for washing and postoperative recovery. A physiological laboratory contains, in addition to standard equipment, a floor trench with standard services, a wall panel for the mounting of heavy apparatus and A.C. electrical outlets for use with a portable roentgen-ray machine. There are four rooms for animals, varying in equipment according to their intended use, but conforming to the specifications of the rooms in the wings used for stock animals. There is a central office and locker room for the men in charge of the animals, a food preparation room with an adjoining cold room and a storage room for grain, hay and shavings. The incinerator room, still room, and a cold room for gross specimens are also included in this unit.

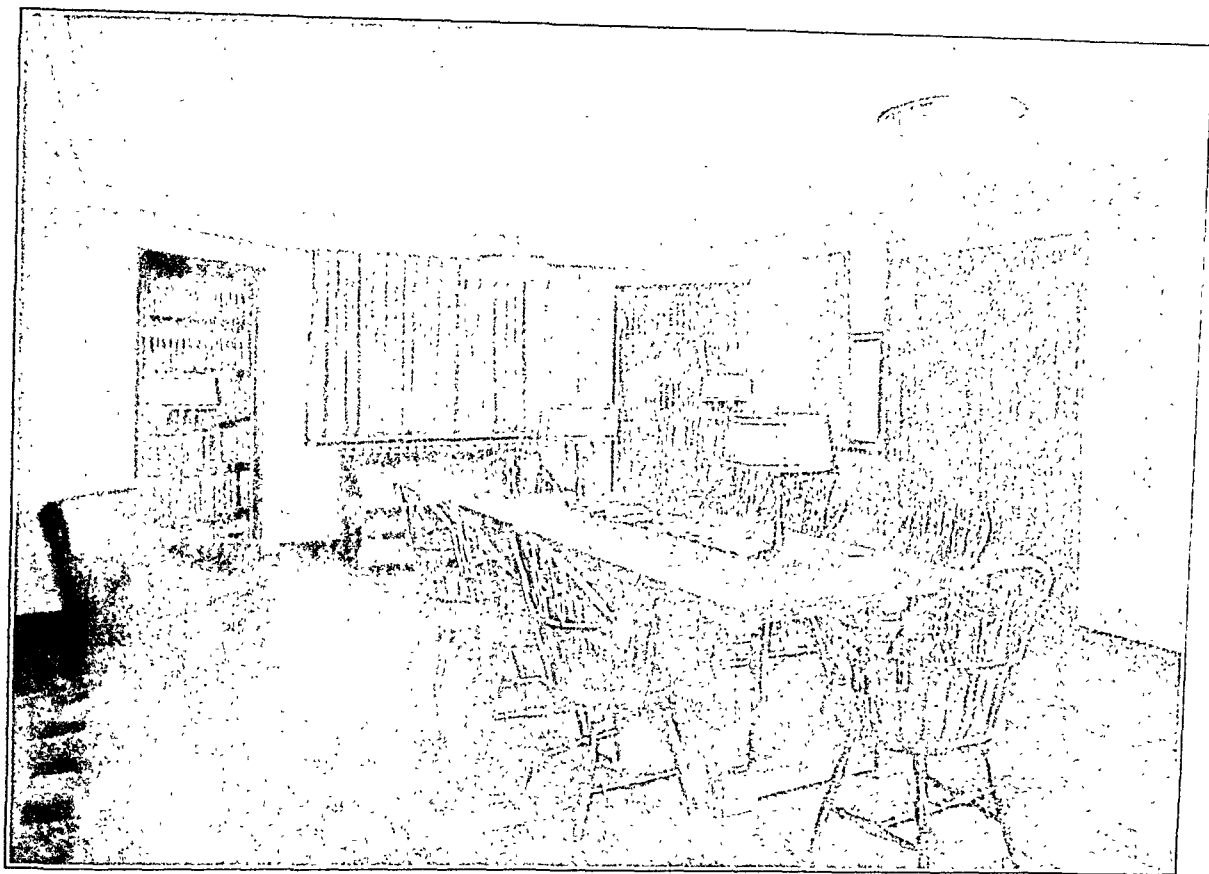
As a fitting recognition of the services that Dr. Frank Burr Mallory has rendered to the Boston City Hospital since the opening of the old Pathological Laboratory in 1895, the Trustees have named this building the Mallory Institute of Pathology. Its purpose is to conduct the routine pathological and bacteriological work of the Boston City Hospital and to investigate the cases referred to the medical examiner's office of Suffolk County, South, both in conjunction with undergraduate and graduate teaching.

Dr. Mallory, as consulting pathologist, continues on with the same activity and interest that he has always shown and his example and guidance serve as an ideal stimulus to the staff.



THE MAIN AUTOPSY ROOM





THE LIBRARY



THE ROOM FOR SURGICAL PATHOLOGY

CONTROLLED FORMATION OF COLLAGEN AND RETICULUM.
A STUDY OF THE SOURCE OF INTERCELLULAR SUBSTANCE
IN RECOVERY FROM EXPERIMENTAL SCORBUTUS *

S. BURT WOLBACH, M.D.

(From the Department of Pathology, Harvard University Medical School, Boston, Mass.)

Previous studies ¹ showed that in the state of complete or absolute scorbutus formation of intercellular materials cannot take place, and that following the administration of antiscorbutics easily demonstrable amounts of intercellular substance are formed within 24 hours. This rapid formation of intercellular substance occurs in regions where mesenchymal cells have accumulated in continuation of normal growth activities, in repair of spontaneous lesions in consequence of scorbutus and in the organization of blood clots after excision of tissue. These facts have made it possible to obtain and to recognize, in preparations from regions of growth and repair, intercellular substances deposited at will through the administration of antiscorbutics. Furthermore, we are enabled to locate exactly where collagen forms in relation to cells and fibrin and to follow the sequences in morphology of intercellular substances to the final or mature state.

Inasmuch as there are several conflicting beliefs concerning the source of collagen and its relation to cells this communication is restricted to observations that are of value as premises for the solution of this problem.

Although a large amount of material from many guinea pigs was on hand, covering all stages in the repair of spontaneous lesions in scorbutus, a new series of guinea pigs was prepared in order to take advantage of the isolation of connective tissue cells which occurs in the avascular organization of blood clots in animals in absolute scorbutus. Absolute scorbutus we define as the stage in experimental scorbutus in which formation of intercellular materials either in growth or repair has ceased. Proliferative reparative cellular responses, epithelial and mesenchymal, are active. It is always accompanied by marked osteoporosis.

* Received for publication September 10, 1933.

The diet was that previously employed by Wolbach and Howe.¹ Guinea pigs of from 315 to 400 gm. initial weight were used. On the 23rd day of the vitamin C free diet a large piece was removed from the extensor thigh muscles of each animal, under aseptic precautions, and the skin incision sutured. The ensuing blood clot was allowed to undergo organization for periods of from 5 to 8 days before administration of orange juice. The duration of treatment with orange juice, before sacrificing the animal, varied from 24 to 96 hours. Control guinea pigs, *i. e.*, those that received no orange juice following the removal of muscle, were killed at the end of 7 to 10 days. From each animal, immediately after death, the experimental wound was excised, with the minimum amounts of surrounding normal tissues, so as to ensure the best possible fixation. The costochondral junctions were also saved and sectioned. Zenker's fixative was used for both types of material. Twenty-four hours fixation was sufficient to decalcify the osteoporotic ribs. All tissues were embedded in paraffin. The stains employed were a modified Giemsa stain² for general study, Mallory's phosphomolybdic acid anilin blue- acid fuchsin- orange G connective tissue stain,³ Mallory's phosphotungstic acid hematoxylin,³ and Foot's modification of the Bielschowsky-Maresch silver impregnation method⁴ followed by Van Gieson's acid fuchsin-picric acid stain, or by Mallory's connective tissue stain.

The repair of the blood clot in absolute scorbutus begins promptly by the migration of fibroblasts from adjacent tissues into the clot, and the continued division of these cells. The fibroblasts are unaccompanied by collagen formation and they retain shapes resembling those of embryonic connective tissue cells, or fibroblasts in tissue plasma cultures (Fig. 1). Capillaries do not penetrate the clot for any considerable distance and are apparently unable to form, although closed columns of endothelial cells accompanied by fibroblasts do form. Islands of erythroblastic cells appear in the peripheral zone of the clot and in the adjacent tissue about capillaries, and apparently are derived from vascular endothelial cells whose initial reparative response to the experimental wound is multiplication. The fibroblasts apply themselves closely to fibrin when the latter is present. They probably follow fibrin strands in their movements (Fig. 2). The behavior of the cells invading the blood clot and the

microscopic picture they produce are similar to those arising in tissue plasma cultures. Fibroglia fibrils are present and stain sharply in absolute scorbutus (Figs. 2 and 9). No collagen or reticulum can be found in any attempt at repair during absolute scorbutus. As shown in Figure 2, the fibroblasts in such granulation tissue are never in close contact and very frequently they are separated by vacuoles outlined by very delicate acidophilic material which is often granular. This appearance of edema is also present in the *Gerüstmark* zone of the costochondral junction and in clusters of fibroblast-like cells on the internal surface of the resorbing cortical bone of ribs in scorbutus. In preparations stained with Mallory's connective tissue stain there is often a faint bluish coloration to the cytoplasm of fibroblasts in organizing blood clots in scorbutus and to the peripheries of the vacuoles between cells. The appearances indicate that in absolute scorbutus fibroblasts are responsible for the formation of an extracellular liquid. The fibroblasts (Fig. 2) themselves are vacuolated usually at their extremities, particularly at points of divergence of fibroglia fibrils, and a frequent picture is that of fibroblasts with spongy cytoplasm whose boundaries are delineated only by the aid of the fibroglia fibrils upon their surfaces. These appearances suggest that the contents of these vacuoles are discharged and accumulate to some degree between the cells. It is to be remembered that these observations apply to cells in regions remote from blood vessels and lymphatics, so that transudation as a source of edema is probably excluded. There can be little ground for denying that the extracellular liquid has its source in the cytoplasmic vacuoles. The vacuoles may be evidence of degeneration or the consequence of excessive secretion of an abnormal cell product; this study supports the latter interpretation.

The presence of collagen following administration of orange juice (10 to 15 cc. daily) was studied at 24, 48, 72 and 96 hour periods. For the sake of brevity the term collagen is used to include the material first deposited around fibroblasts in repair, including the intercellular material first laid down in the formation of bone. It includes so-called reticulum (argyrophile fibrils), as determined by silver impregnation methods, as well as the material having the normal staining properties of collagen with the methods employed in this study. Collagen and reticulum were found around cells, often completely isolated, far from blood vessels and preëxisting normal

tissues. The deposit of collagen appeared later and less abundantly about those cells that had penetrated farthest into the blood clot, so that deposits of different ages could be identified in single preparations. The collagen was found only in immediate contact with cells. The distribution of the first deposit is determined by the shape of the cell and influenced by the proximity of other fibroblasts. The collagen about isolated cells, far in the blood clot, surrounded the individual cell and did not radiate or extend into the clot away from the cell. The same applies to fibrils impregnated with silver by Foot's modification of the Bielschowsky-Maresch method. This restricted localization of the collagen was most strikingly evident about rounded, non-processed fibroblasts that had come to rest deep in the clot (Fig. 4).

Elongated cells with processes showed collagen distributed in all the directions of the cell processes and as far as the fibroglia fibrils extended. Elongated cells in contact with fibrin showed collagen on all sides of the cell (Fig. 3), and the fibrin remained unaltered during the early periods of collagen formation.

No appearance remotely suggestive of the transformation of fibrin into collagen was found, or that fibrin contributed in any manner to collagen formation. One observation which might possibly be construed as evidence for a participation of fibrin in collagen formation is that excavation of large fibrin masses by fibroblasts is more conspicuous after collagen formation has been induced (Figs. 5 and 6). This, however, may be explained by the restoration of enzymatic functions to the cell. There is evidence that the rôle of the fibroblast and osteoblast in collagen formation is a reversible one, for the collagen deposited in the early repair of bone in recovery from scurvy may later be resorbed about these same cells. Whether or not the products of fibrin digestion assist in the formation of collagen, beyond providing sustenance to the cells, cannot be decided by these experiments, although it may be positively asserted that fibrin is not essential to collagen formation and that direct transformation into collagen (or reticulum) does not occur.

The first material having the staining properties of collagen which appeared around cells was homogeneous, resembling lightly stained amyloid and therefore presumably not of great density. It was best observed where fibroblasts were grouped closely together and between fibroblasts and fibrin strands (Figs. 3 and 5). This homo-

geneous state was of short duration, though best seen on the second day when collagen deposition was rapid; no extensive zones of it were found. It was intimately applied to the cytoplasm of the cell body or to fibroglia fibril extensions beyond apparent limits of the cell. Its site of formation in relation to the cell body was always external to fibroglia fibrils. It was present about cells when no reticulum or argyrophile fibrils could be demonstrated.

Next in sequence was the appearance of delicate fibrils coincidentally with the appearance of reticulum, or argyrophile fibrils (Figs. 5 and 6). With isolated, rounded or ovoid cells the direction of the fibrillary collagen, as well as that of argyrophile fibrils, was concentric to the cell body; if the cells were processed the fibrils paralleled the cell processes and fibroglia fibrils. In cell clusters the fibrils coursed irregularly between cells and it was impossible to associate any group of fibrils with a definite cell (Fig. 7).

Early in recovery from scorbutus, up to 72 hours (Fig. 6), the reticulum method gave the appearance of there being exceedingly delicate black fibrils embedded in a homogeneous collagen matrix, producing the effect of broad bands of black reticulum. This microscopic sequence may be explained as fibril formation in a homogeneous material. More mature collagen in later periods of recovery does not impregnate with silver. The first detectable argyrophile material appears in the form of black granules at the limit of microscopic resolution, arranged in rows, always somewhat tortuous, never straight. Identical sequences were found in the *Gerüstmark* zone of the ribs and in the resumption of osteoid deposit in the endochondral formation of bone (Fig. 8) and upon the inner ("endosteal") and outer (periosteal) deposition of osteoid.

The conclusion is unavoidable that the earliest formed collagen fibrils are identical with reticulum and that the property of impregnating with silver is due to delicacy of the fibrils, a conclusion reached by Mallory and Parker ⁵ from a study of human tissues and tumors, by Alfejew ⁶ from the study of mammalian embryos, and by Maximow ^{7, 8} from the study of rabbit tissue plasma cultures.

In the resumption of bone formation in recovery from scorbutus sequences identical to those described above take place, and the first deposit of intercellular material cannot be differentiated from that which forms in organizing blood clots and in other regions not destined to ossification.

The study of bone resorption in regions of most marked osteoporosis in absolute scorbutus offers convincing evidence that some liberated bone cells survive and increase in size and acquire appearances usual to fibroblasts. Fibroglia fibrils can be seen in enlarged bone canaliculi and to extend into the enlarged marrow cavity. Traces of matrix extending inward from the cortical bone lie parallel to fibroglia fibrils. The lacunae in which bone cells lie are enlarged (Fig. 9). These facts are mentioned because they are some of the observations that indicate resorption of bone matrix and its collagen constituent is dependent upon the activity of those cells that under other conditions are concerned in its formation. They also indicate that the fibroglia fibrils are conductors of the agents concerned in resorption as well as in the deposition of matrices, and that fibroglia fibrils may have a cytoplasmic vesture. In recovery from scorbutus (Fig. 10) prompt formation of intercellular substance at first indistinguishable from collagen by reticulum staining methods and the usual connective tissue stains took place. As in the case of collagen formed in the organization of blood clots the deposit in bone formation was at first homogeneous, then fibrillary, and its distribution determined by cells and their processes. Studies which will not be presented here support the belief in the existence of a homogeneous material enveloping the collagen fibers in the completed bone matrix.

SUMMARY AND DISCUSSION

The organization of blood clots during the state of absolute scorbutus in guinea pigs is a process in which fibroblasts become widely separated from their sources and from blood vessels, capillaries and lymphatics. Fibroblasts may also be found separated from one another, migrating and dividing as in tissue plasma cultures, following strands of fibrin and penetrating masses of blood corpuscles in regions where stainable threads of fibrin are absent. Collagen does not appear either as reticulum or in more easily demonstrable form until recovery is induced by the administration of an antiscorbutic. Under the conditions of these experiments the exact situation of reticulum and collagen formation has been determined.

Collagen appears first as a homogeneous material in which argyrophile or reticulum fibrils promptly appear. Coincidentally with the appearance of the argyrophile fibrils the stains in common usage for

demonstrating collagen show the presence of collagen fibril bundles. The distribution of the collagen is dependent upon the form of the cell and with isolated cells is confined to zones immediately adjacent to the cell body and its processes, including the entire length of fibroglia fibrils.

The course or direction of the collagen and argyrophile fibrils is parallel to surfaces of the fibroblast and its processes. Because of this arrangement parallel to the cell and its processes, and never radiating, fibril formation must be influenced by factors not present in the formation of fibrin strands from plasma, and speculation is suggested concerning a molecular alignment previous to the development of fibrils. Further speculation suggests that the pattern of collagen fibrils formed rapidly in groups of cells which are without processes is influenced by many cells as by a resultant of forces acting in the homogeneous or amorphous stage of collagen formation. Where the processes and fibroglia fibrils of fibroblasts interweave the appearances are that each cell is accompanied by collagen fibrils in parallel arrangement to its ramifications.

Careful study of regions containing fibrin in experimentally produced clots and in regions of spontaneous injury, particularly at the line of infraction in ribs, yielded no suggestion of transformation of fibrin into collagen. The presence of fibrin does not modify the arrangement of collagen about the fibroblasts, which is conclusive evidence that fibrin does not directly contribute to the formation of collagen.

Baitsell,⁹⁻¹² both in tissue plasma cultures and in the formation of tuberculous tissue in the guinea pig testis, has presented evidence for the transformation of fibrin and exudate into collagen. His material was so different from that of the present study that it is impossible to exercise critical judgment in comparison of conclusions. It may be said that the conditions in tuberculous testes of guinea pigs are exceedingly complex, as compared to the recovery phenomena in organization of blood clots in scorbutus.

Nageotte,¹³ in many types of experiments, concludes that fibrin is transformed into collagen, but also states that other albuminoid substances, including the protoplasm of dead cells, may be precursors of collagen.

In embryos a common and well substantiated origin of collagen is "a transparent, gelatinous, cell-free, ground substance which, in

general, pervades the embryonic body from very early stages of development."¹⁵ The recent papers of Baitsell,^{14, 15} Harrison,¹⁶ and Alfejew⁶ all express the opinion that fibrils form in this ground substance independently of the immediate proximity of cells. Maximow and Bloom⁸ describe spiral argyrophile fibrils formed in tissue cultures remote from cells. This observation seems to have been made by Bloom, as it does not appear in Maximow's publication⁷ based upon the same material.

An early conception of the origin of collagen fibrils was the direct modification of the cytoplasm of the surface of the fibroblast and its processes, held by Hansen,¹⁷ and Mall,¹⁸ and other noted investigators. The most substantial recent support of this view is that of M. R. Lewis¹⁹ from tissue culture studies. According to Lewis the fibrils appeared in cultures after 24 hours growth, "as slightly more refractive lines within the cytoplasm of the individual cell" and later became gathered into bundles outside of the cell to form slender fibers. Mitochondria were described as being occasionally carried by fibrils to points outside of the cell, though not concerned in fibril formation, as has been held by Meves,²⁰ and others. The conclusions of this impressive paper cannot be reconciled with those of the present study. A further divergence is Lewis' conclusion that the vacuoles which form in fibroblasts are not concerned in fibril formation, as first suggested by Péterfi²¹ in the study of argyrophile fibrils in epithelial cells of the amnion of fowl embryos.

Whether or not the vacuoles that form in fibroblasts in the scorbutic guinea pig have a counterpart in fibroblasts in granulation tissue under normal conditions has not been determined. Their presence in scorbutus may be regarded as pathological, but also as due to an exaggeration of a normal process, and in all probability as the source of the extracellular liquid which is so conspicuous in certain regions in experimental scorbutus and which may represent a liquid precursor of collagen, as suggested by the rapid appearance and large volume of intercellular material in the recovery from scorbutus.¹

In the above discussion reference has been made only to papers with a bearing upon observations made in the present study. Excellent reviews covering the literature of collagen formation are to be found in Lewis' paper¹⁹ and by Maximow in Möllendorff's *Handbuch*.²² Relevant to the subject, as concerning intercellular substances, are the recent articles with exhaustive reviews of the

literature on cartilage by Schaffer,²³ and on bone by Weidenreich,²⁴ also in Möllendorff's Handbuch.

Consideration of the three theories held in explanation of collagen fibril formation — (1) the intracellular by transformation or metaplasia of the surface cytoplasm (ectoplasm) with or without the participation of mitochondria; (2) the origin in an extracellular amorphous ground substance secreted by cells, and (3) the transformation by enzymes of extracellular material not produced by fibroblasts, such as fibrin or other forms of exudate and albuminoid materials from dead cells — shows compatibility of only the second with the results of the present study. In embryos large amounts of amorphous precollagen are formed and fibril formation apparently takes place in regions remote from cells. In Maximow's tissue cultures the first evidence of intercellular materials was the formation of argyrophile fibrils, apparently not necessarily in close contact with cells and extending into the medium without relation to cell processes.

In recovery from scorbutus the collagen deposit is restricted to the immediate vicinity of fibroblasts. It is at first amorphous. Argyrophile fibrils appear first in the earliest deposited material; this can be best seen in the resumption of bone matrix formation in endochondral bone formation (Fig. 8). About fibroblasts the collagen distribution is definitely related to the cell body and its processes, including the fibroglia fibrils. The study of resorption of bone and the shapes assumed by released bone cells indicates that the fibrils of the latter are identical with fibroglia fibrils and that they in some manner are active in the resorption of bone matrix. The distribution and directions of collagen fibrils in recovery from scorbutus are also influenced by fibroglia fibrils. In addition to possible mechanical functions fibroglia fibrils are conductors of agents presumably having reversible enzyme activities.

The writer's evaluation of the conclusions drawn from this study is influenced by unpublished studies of sequences in resumption of growth of bone in recovery from rickets, scorbutus and vitamin A deficiency. In each instance the reparative processes were identical with the normal sequences that the deficiency had suspended, but they proceeded, in their early stages, at a rate far beyond that of normal growth. This is probably true of recovery phenomena from all vitamin deficiencies and seems definitely so in the resumption of collagen formation by fibroblasts in the present study.

CONCLUSIONS

1. Fibrin and other preformed materials do not contribute to collagen formation in repair by organization.
2. Collagen and reticulum represent physical differences of the same material.
3. Collagen is the product of secretory activity of fibroblasts, and its alignment and distribution are determined by the shape of the cell and its processes, including fibroglia fibrils.

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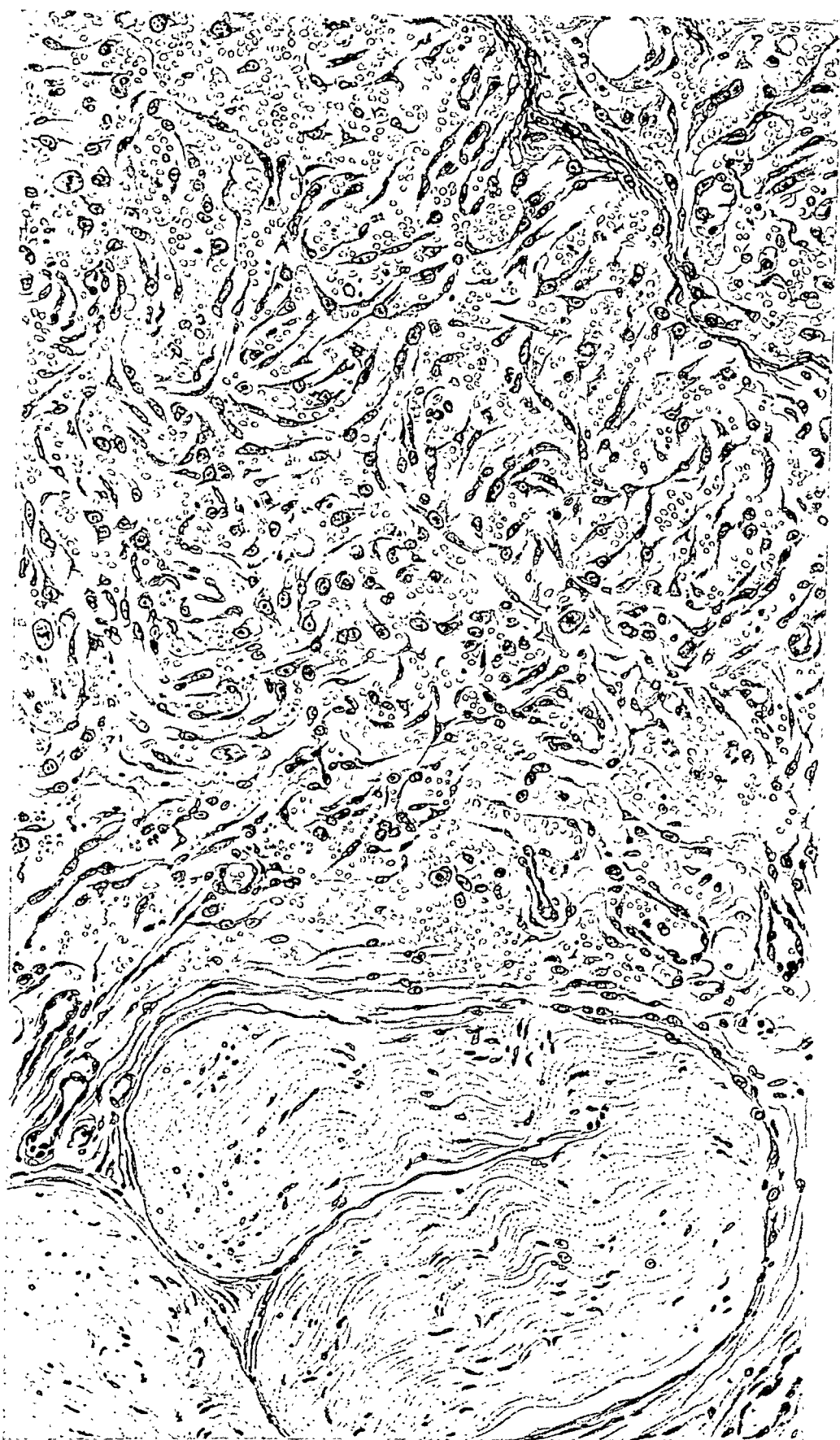
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DESCRIPTION OF PLATES

PLATE 112

FIG. 1. Repair by avascular organization in absolute scorbutus. A strand of fibrin crosses the upper right-hand corner. Most of the cells in this field have probably emigrated from connective tissue surrounding the nerve shown in the lower border. Most of the fibroblasts are separated from one another. Five mitotic figures are in this field. Guinea pig operated upon on 23rd day of vitamin C free diet; killed on 30th day of deficiency. Initial weight 315 gm., maximum weight 360 gm., and final weight 204 gm. Modified Giemsa stain. $\times 125$.



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PLATE 113

FIG. 2. Fibroblasts in relation to fibrin strands in organization in absolute scorbutus. Fibroglia fibrils are prominent. Note vacuoles in cytoplasm of the fibroblasts and extracellular vacuoles described in the text. No stainable collagen, including argyrophile fibrils, present. Guinea pig operated upon on 23rd day of deficient diet; killed on 33rd day of the deficiency. Initial weight 400 gm., maximum weight 446 gm., final weight 320 gm. Mallory's connective tissue stain. $\times 1000$.

FIG. 3. To illustrate the collagen formed in organization tissue during a 40 hour period of recovery from absolute scorbutus. The region illustrated is deep in the blood clot. Note homogeneous appearance of the collagen surrounding cells in contact with fibrin in left third of the field; fibrillation of collagen is apparent elsewhere. Other preparations stained by Foot's method showed argyrophile fibrils in small numbers and corresponding to the fibrils illustrated. Guinea pig operated upon on 23rd day of deficient diet; orange juice administered on afternoon of 28th day; killed on morning of 30th day of the experiment. Initial weight 357 gm., maximum weight 395 gm., final weight 301 gm. Mallory's connective tissue stain. $\times 1000$.

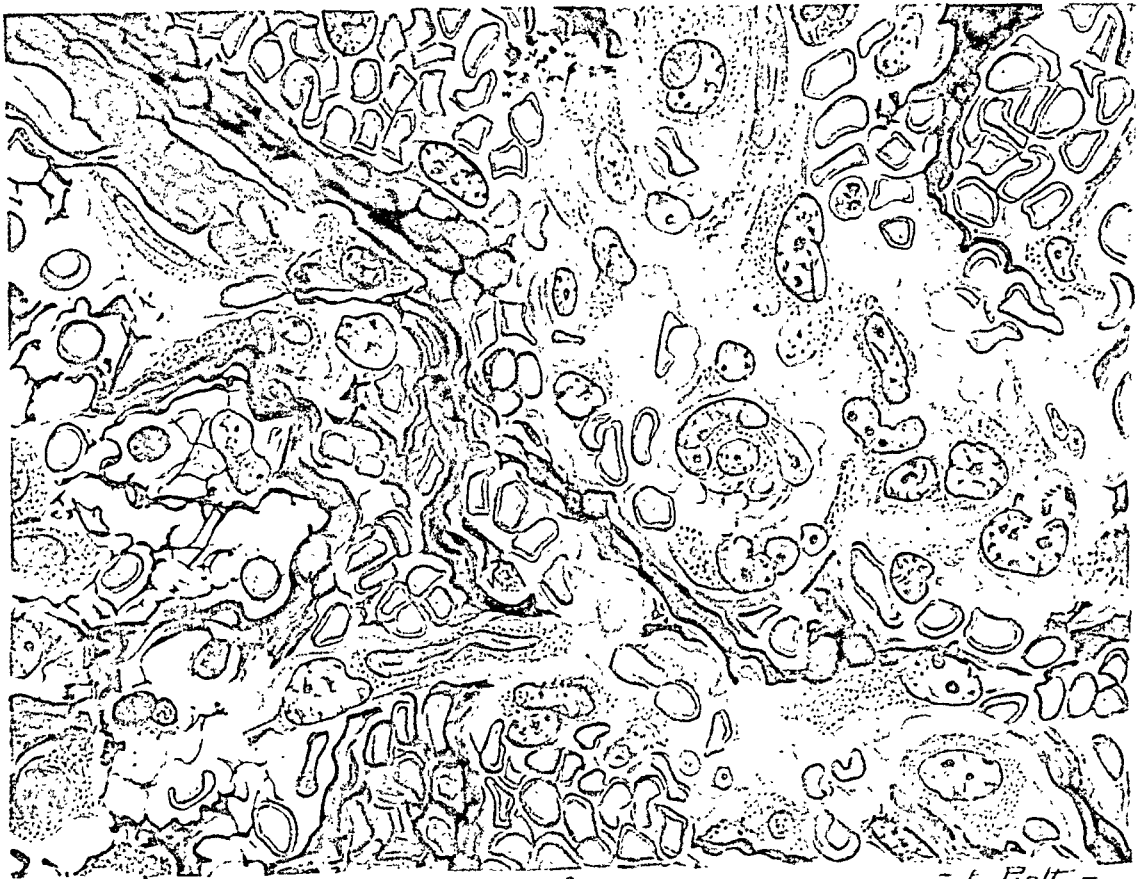
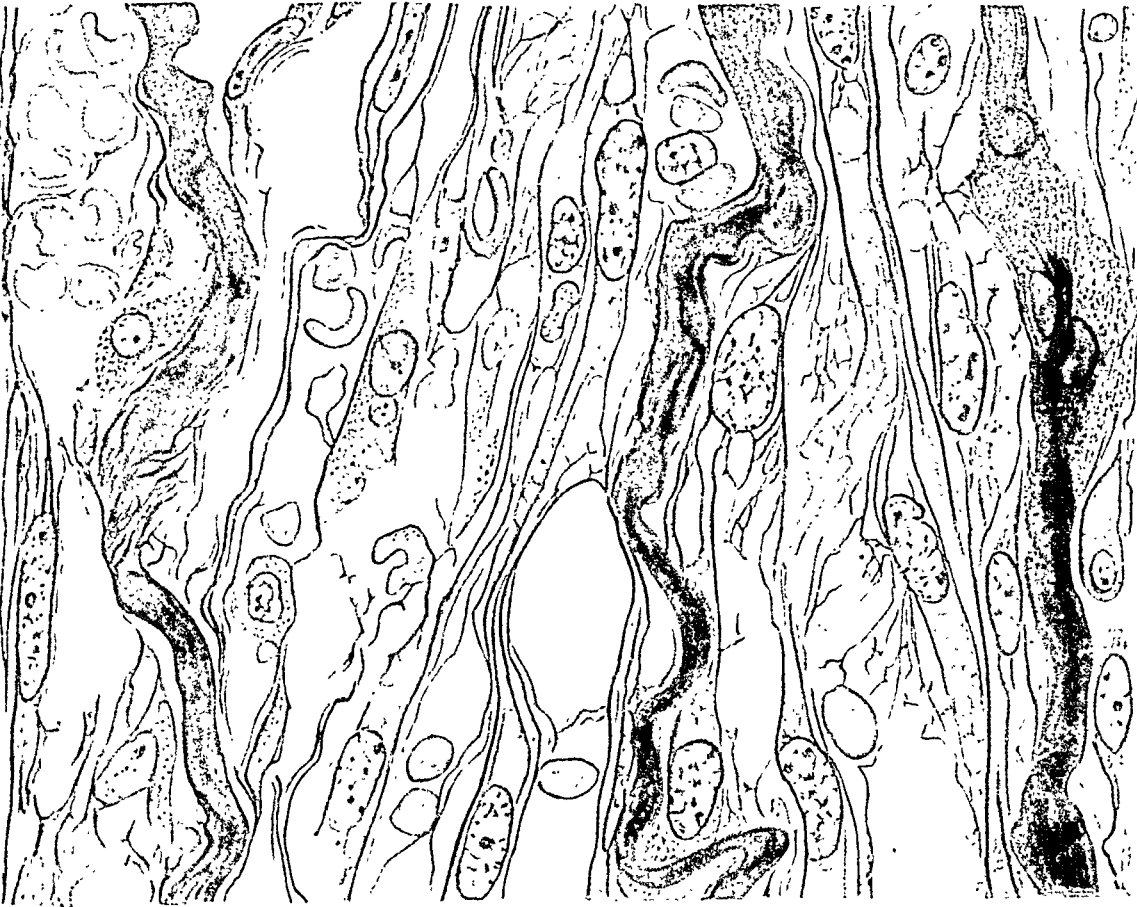
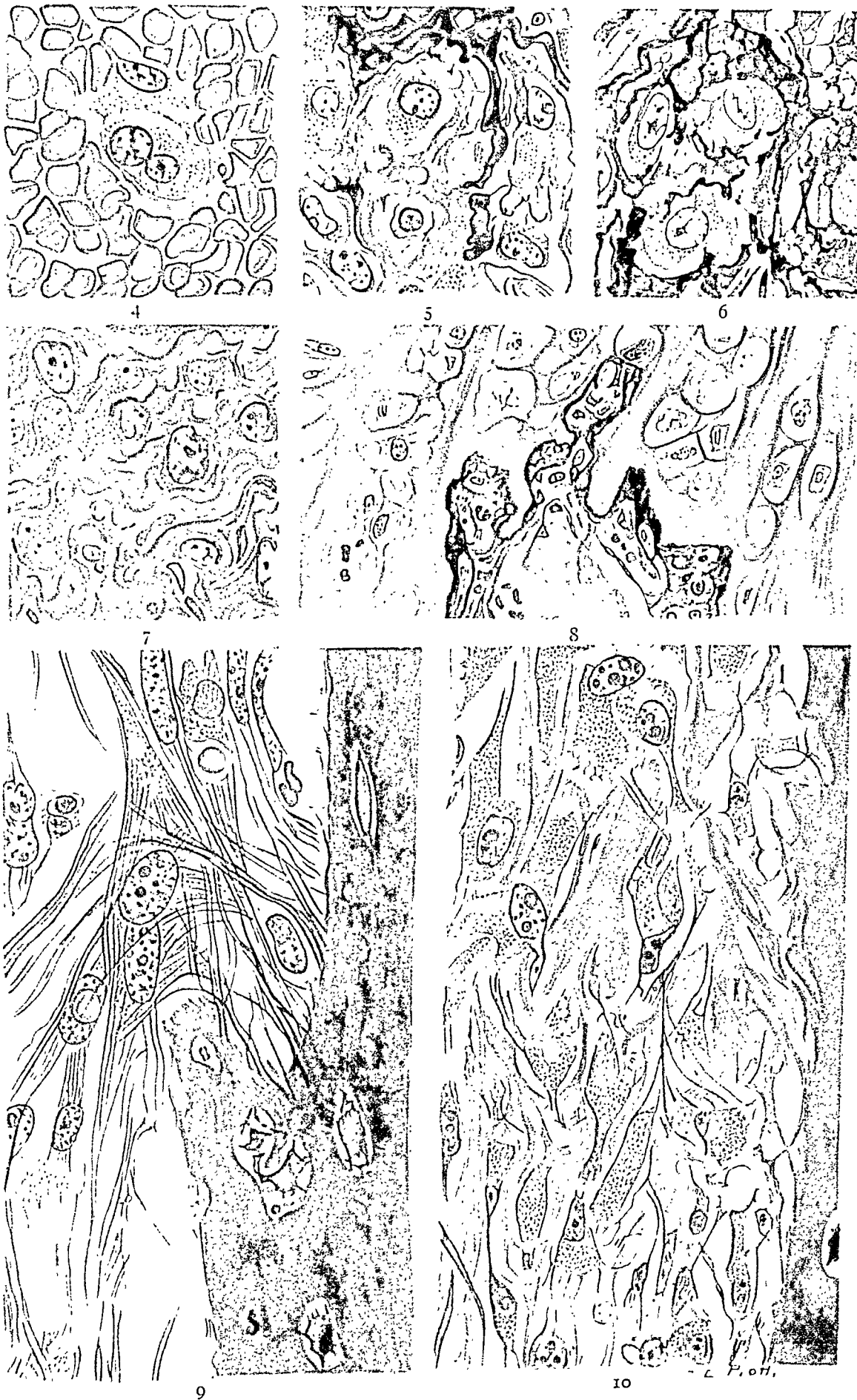


PLATE 114

- FIG. 4. To show collagen in relation to an isolated cell deep within the blood clot. Recovery period of 72 hours. Guinea pig operated upon on 23rd day of deficient diet, orange juice first given on 31st day of experiment. Initial weight 395 gm., maximum weight 418 gm., final weight 288 gm. Mallory's connective tissue stain. $\times 1000$.
- FIG. 5. Recovery period of 72 hours; same animal used for Fig. 4. A field from a group of cells deep within the clot, illustrating collagen deposited by cells that have penetrated fibrin strands. Mallory's connective tissue stain. $\times 1000$.
- FIG. 6. Recovery period of 72 hours. This field corresponds to that of Fig. 5 and is from the same block at a slightly different level. The argyrophile fibrils have formed and their distribution in relation to the cells is the same as that of the collagen. Foot's modification of Bielschowsky-Maresch silver impregnation method, followed by Mallory's connective tissue stain. $\times 1000$.
- FIG. 7. To illustrate collagen formed by a cluster of cells during a 96 hour repair period. Guinea pig operated upon on 23rd day of deficient diet; orange juice administered from 31st day to 35th day inclusive; killed on 36th day of experiment. Mallory's connective tissue stain. $\times 1000$.
- FIG. 8. To illustrate resumption of endochondral bone formation. Rib, recovery period of 40 hours duration. Preparation from the same guinea pig used for Figs. 5 and 10. To illustrate newly formed argyrophile fibrils in presence of homogeneous collagen representing the first stage in formation of the intercellular substance of bone. Argyrophile fibrils have formed in the amorphous collagen first deposited by the immature osteoblasts. Staining technique same as that of Fig. 6. $\times 430$.
- FIG. 9. "Endosteal" surface of rib of guinea pig in absolute scorbutus. To illustrate details of bone resorption described in the text. Note the appearances of cells indistinguishable from fibroblasts which in recovery from scorbutus become osteoblasts. The yellow mottling of the bone is characteristic of advanced scorbutus, when stained by Mallory's method. Note the continuity of fibroglia fibrils emerging from the cortex with the fibroblasts in the cavity of the bone. Mallory's connective tissue stain. $\times 1000$.
- FIG. 10. To illustrate early "endosteal" bone formation during a 40 hour recovery period and relation of the collagen to cell shapes and fibroglia fibrils. Rib, from guinea pig used for Figs. 3 and 8. Mallory's connective tissue stain. $\times 1000$.



THE CANCER CELLS OF SEROUS EFFUSIONS *

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Free tumor cells appear commonly in the fluid accumulations consequent upon neoplastic disease of the serous cavities. Their recognition is of practical importance through its value in facilitating clinical diagnosis in cases otherwise obscure. These cells are also of much interest from the standpoint of general cytology and oncology. Known for many years, they have been studied for the most part only in smear preparations or in the wet state. The resulting uncertainties have bred a laboratory tradition highly suspicious of any attempt at their positive identification. They may, however, be seen clearly in preparations secured by centrifugation of fresh aspirated fluid, followed by Zenker fixation, paraffin embedding and staining of the centrifugate. This simple method was discovered a number of years ago. It appears to have been developed independently by several workers¹ and the general procedure was described by Mandlebaum² as long ago as 1917.

The present observations are based upon the study of some fifty cases of implanted or metastatic pleural or peritoneal carcinoma encountered in the course of routine diagnostic work during the past ten years. The preparations have been stained by hematoxylin and eosin or eosin-methylene blue, supplemented at times by iron hematoxylin or Mallory's phosphotungstic acid hematoxylin and aniline blue connective tissue stains. The material has been prepared and fixed as soon as possible after its withdrawal from the body, but cell preservation has been surprisingly good even in some fluids fixed 24, or even 48 hours later. Diagnostic findings have been obtained occasionally on small amounts of the aspirated fluid. In some cases as little as 10 to 15 cc. have provided a centrifugate mass permitting definite conclusions.

Several types of cell arrangement are present in the different fluids. Easiest of recognition as products of neoplastic proliferation

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are microscopic fragments of actual tumor tissue. Particles with epithelium and stroma have been seen in three cases, two of them presenting obvious papillary structure. In two other cases there are disintegrating remnants of colloid carcinoma. These are the only instances in which stroma elements are found in association with epithelium. In a peritoneal effusion due to gastric carcinoma there are a few groups of epithelial cells unaccompanied by stroma but showing plainly a suggestion of alveolar arrangement, while traces of mucus cling to the walls of the suggested lumens. In three fluids from the pleural cavity, one following bronchiogenic carcinoma, and two consequent upon metastasis from mammary carcinoma, there are large clumps of cells which, in the sectioned preparation, have a pavement-like arrangement of large units, the picture suggesting squamous epithelium. Cytoplasmic and nuclear structure are sufficient to establish the conclusion that these have nothing to do with the benign "endothelial plaques" of the early French writers. In each case there is the confirmatory evidence of clear-cut mitotic figures.

Mitosis may be infrequent or very prominent (Fig. 4). It is found in isolated cells as well as in individual elements of cell groups. Its occurrence, together with other evidences of good cell preservation and the peculiar organoid arrangements sometimes encountered, is believed to indicate that the neoplastic cells are proliferating within the serous fluid and not merely floating in it as elements desquamated from the surface of mural tumor masses. It may be assumed that these free cells are to some extent removed from the regulating influence of such factors of tissue balance as may apply to growth within the solid tissues. On the other hand, they differ from the cells of artificial tissue cultures in possessing nutritional and environmental conditions more nearly approaching the normal than is possible for cells growing *in vitro*. They might, therefore, be expected to provide an opportunity for the study of tumor cells from an angle somewhat different from the usual.

In several of the fluids there are formations that show very clearly their descent from glandular epithelium. Three of the six instances encountered were ascitic effusions following peritoneal implantation of cystic adenocarcinoma of the ovary and a fourth was probably of ovarian origin, although laparotomy failed to reveal the original tumor site. Another peritoneal effusion followed metastasizing

adenocarcinoma of the fundus uteri. One well defined example occurred in a pleural effusion, but unfortunately the site of origin of the tumor could not be checked by autopsy. These fluids contain aggregations of epithelial cells free of stroma but exhibiting distinct adenomatous arrangement. Some of the groups may appear as clumps of columnar elements with disintegrative changes in the centrally located cells. Many are hollow vesicles, the cavity empty or containing a few loose cells and occasionally colloid droplets or a faint network or linear pattern of fine lines suggesting the presence of mucinous residue. In one specimen the prevailing arrangement is that of clear-cut epithelial circlets with relatively large clear central spaces. Serial sections prove them to be hollow spheres or vesicles with a single layered wall that may, however, contain occasional outward-bulging clumps of irregularly arranged cells (Fig. 1). The cells of the vesicle wall may be flattened, cuboidal or polygonal and occasionally distinctly columnar. They may be well preserved or vacuolated. Wide variations in morphology occur even within the individual vesicle. Along with the vesicle type of formation there appear in one specimen groups of columnar cells in acinar or tubular arrangement of strikingly organoid appearance. The organoid structure is particularly marked in one specimen where many of the groups contain one to several mitotic figures. The vesicle arrangement was seen in one case in the wet unfixed material. It was noted long ago by Ehrlich and by Beneke,³ but the wet preparation or smear gives only a very imperfect idea of the structural details.

In over half the cases of the series the tumor cells occur singly or in small groups of only a few units (Fig. 2). Here, also, their neoplastic character may be clearly evident but occasionally becomes difficult of demonstration. The difficulty lies in the long recognized problem of their differentiation from the large cells seen in fluids of neoplastic as well as inflammatory origin and usually accepted as mesothelial. The identity of these cells needs further working out by modern methods. As they are observed in some cases in close association with tumor cells and equalling, or even exceeding them in number, the following question arises. Are they stimulated to overproduction by the tumor growth, or is the relation reversed and do they serve like Carrel's "nurse cells" of tissue cultures⁴ to provide food or activating factors for the neoplastic cells? As a matter of practical differentiation, it is only rarely that careful search of the

transudate resulting from neoplastic growth will fail to reveal single cells, or cell aggregations that betray their tumor origin, even when these cells are far outnumbered by leukocytes, or by elements of doubtful type. Divergence from the normal takes the direction of irregularity in size, and particularly of increased size of the cell, enlargement and hyperchromatism of the nucleus, and increased prominence of the nucleolus. Mitosis is, of course, highly significant. It is doubtful whether this occurs in other than neoplastic cells with sufficient frequency to complicate the problem, despite such conclusions as those of Karp,⁵ who found it often in cells accepted as mesothelial in smear preparations. Fat droplet accumulations and hydropic or other vacuolation and disintegrative changes indicative of metabolic disturbance may be common in tumor cells as well as in the other types present. Coarse vacuolation often results in the formation of signet ring figures. The signet forms of the neoplastic cells may consist of two or more cells and, as believed by Karp and previous observers, tend to be larger than those of mesothelial origin.

A common finding, which remains as difficult of interpretation as it was in the early days of pathological cytology, is the incorporation within the cytoplasm of a tumor cell of one or more other cells, themselves definitely neoplastic. These are the mother cells, regarded by Virchow as producing daughter cells by endogenous proliferation. The included cells have been considered, on the other hand, merely as invaginations. As seen in the present preparations, they seem to be definitely within and not merely pressed against the larger cytoplasmic mass. Within the solid tissues such forms might readily be interpreted as the result of mutual pressure, but such origin is not likely here. By contrast, leukocytic invasion of the cytoplasm is uncommon. The included cells may be closely enveloped or may lie in a vacuolar space. While often well preserved, or even in mitosis, they are apt to be small, with more or less evident cytoplasmic rarefaction and disintegrative nuclear changes. The including element may be a single cell of natural size but it is commonly large with multiple nuclei or a heavily chromatic or multilobulated single nucleus (Fig. 6). The included material may consist of a clump of two or three cells or may itself appear multinucleated and even of giant cell type. The suggestion is strong that the included cells have been taken up by hardier individuals to be used as a

direct source of nutrient material, or to be incorporated directly into their own substance. The phenomenon may, in fact, be allied to that of cell fusion.

The question as to whether or not individual cells may under appropriate conditions fuse with other cells of their own type has been a subject of prolonged controversy. Mallory⁶ has long championed such power of coalescence as an explanation for the genesis of the foreign body giant cell, an idea now generally accepted. The early suggestion of Krauss⁷ that a similar fusion may occur between neoplastic cells has not met with acceptance. It was, however, considered favorably by Steinhaus,⁸ who summarizes the literature of a period during which this and allied topics were being actively debated. Haythorn⁹ believes that the giant cells studied by Krauss were actually of foreign body type. Fusion of tumor cells was accepted in the earlier work on the culture of malignant tumors of animal and human origin.¹⁰ Cells showing similar fusion were regarded by Lewis¹¹ as modified macrophages. Carrel and Ebeling⁴ explained as degenerative phenomena the fusion forms, as well as the irregular mitoses and other abnormalities seen in cultures prepared by the earlier methods. Similar criticism does not seem to apply to the syncytia appearing in carcinoma cultures. The cell forms of the present material suggest that fusion is actually taking place and that individual tumor cells merge into larger units. Such fusion is believed to offer one explanation for the occurrence of giant cells.

Giant cells appear in nine of the fluids, sometimes in small, but in several cases in large numbers. In only rare cells does the cytoplasmic and nuclear structure suggest the foreign body type. In some cases there are associated multipolar mitotic figures, but again these may be extremely uncommon, so that but one or two may be found in slides containing large numbers of giant cells. Occasionally there are bipolar spindles with apparent excess of chromosome numbers. In fluids with well preserved cells of regular size there may occur rare giant forms reaching diameters of 70 to 80 microns, with a single, round, sharply contoured or slightly fluted nucleus, and with natural balance between nuclear and cytoplasmic volume. In most cases, however, the nuclear volume tends largely to overbalance the cytoplasmic so that relatively great masses of nuclear material are surrounded by the scantiest zone of cytoplasmic substance. The

nuclei may be single or multiple. Marked lobulation is usual and the nuclear subdivisions are heavily dotted with prominent nucleoli. There is often great variation in the chromatin content of the individual nuclear masses or of the irregular subdivisions of the single lobulated nucleus. Many attempts have been made to explain these curious figures. Their variety is bewildering but it is probable that all depend upon a common phenomenon. It is possible that the multiple forms of equal size and chromatin content may result from multipolar mitosis in a nucleus of giant size, but such forms are the exception rather than the rule. On the other hand, multiple forms of unequal size may result from unequal subdivision of chromatic material when division has been accompanied by the presence of an odd number of centrosomes and spindles. Again it is possible that the lobulation and constriction of the single nucleus and the occurrence of separate nuclear masses of unequal size may best be explained by direct nuclear division.¹² The evidence, however, is believed to warrant the contrary suggestion that these bizarre forms result from a successive building up of larger and more deeply chromatic nuclear formations through a gradual process of cell fusion, such fusion involving not only the cytoplasmic material but the nuclear substance as well. While it is usually accepted that the multipolar mitotic figure is associated with the appearance of tumor giant cells, it is not clear which is primary. The question may still be asked as to whether the multiple spindle formation is cause or effect of the hyperchromatic nucleus, whether this nucleus be single, multiple or lobulated. No satisfactory explanation has been given for the original formation of the giant chromatic aggregation. It has usually been regarded by pathologists as a degenerative phenomenon and there is much to support this belief. It has been ascribed also rather vaguely to fluid imbibition or to hypertrophy from unknown causes. Levine¹³ suggests diffuse foci of exalted intranuclear growth as the origin of lobulation and is inclined to agree with the zoölogists that the giant size may be an evidence of increased growth energy rather than a forerunner of cell decay. The evidence from tissue culture work favors the long held belief that cell fusion, giant cell formation and atypical mitosis depend in the first instance upon factors unfavorable to cell growth, whether these factors be diminished oxygen tension, changes in hydrogen ion concentration, the accumulation of interfering products of cellular

metabolism, or other influences as yet unknown. The association of degenerative changes is well brought out in a recently studied carcinoma of the breast where thick columns of tumor cells in a medullary carcinoma are suddenly replaced in the marginal zone of an area of gross necrosis by columns made up of cells greatly reduced in number but practically all of giant size with great multiple or lobulated nuclear masses. In the fluids under discussion the number and variety of fusion figures and giant cells appear to vary with the degree of the degenerative changes affecting the various cellular constituents.

Granting that giant cell formation in tumors is a regressive change in the sense of its dependence upon unfavorable growth factors, it may still be accepted that for the individual cell it may constitute actually a protective mechanism. It may then be regarded as the result of a determined struggle for existence on the part of the neoplastic cell and as an indication of its ability to build up a volume of nuclear material far beyond the usual requirements.

Figures believed to represent fusion forms may sometimes appear in early stages as cell clumps with a peripheral enveloping layer of flattened elements one cell thick (Fig. 7). The inner cells appear to fuse gradually and, as this takes place, marked irregularity makes its appearance in the size and chromatin content of the individual nuclear masses of the aggregation. There appears to be a condensation of the nuclear material into one or more segments of a coalescing chain, other segments of which are fading out. There may be separate nuclear formations sharply defined or faintly suggested and more or less devoid of chromatic material (Fig. 3). A final stage is represented by a central single, multiple or lobulated, deeply stained nucleus with many nucleoli and faint cytoplasmic investment, outlined by a prominent surface zone of denser cytoplasm, reticulated or granular and often exhibiting many delicate circumferentially arranged fibrils. The cytoplasm may occasionally contain coarse threads of basic-stained material and not infrequently there are faintly defined shadows suggesting nuclei or nuclear remnants (Fig. 8). Mitosis does not seem to play a part in the formation of these masses. In other cases cell clumps of more open type, with irregularly disposed components, may include cells containing bipolar mitoses of normal chromosome content, or such dividing cells may lie within the cytoplasm of giant cells. Here the nuclear irregu-

larity or giant size appears to have antedated the incorporation of the dividing cell. The fate of such dividing elements cannot be made out. It is possible that their mitosis is abortive and their chromatic material, as well as other constituents, is taken up directly and incorporated by the enveloping cell mass.

Multipolar mitosis appears as commonly in association with giant cells as it does in solid tumors, although, as noted, it may be relatively uncommon or practically absent in the individual specimen. The spindle organization may be well defined, but again it may be highly complex and confusing. The details of centrosphere and spindle structure are often vaguely made out and may even completely escape detection. It is extremely uncommon to find anything suggesting reorganization of the chromosomes into daughter nuclei. There can be little doubt that in at least some of the cells of these fluids attempted multipolar mitosis results in cellular disintegration. It is not uncommon to find cytoplasmic masses permeated by swarms of chromosomes with no regularity of size or arrangement and no suggestion of spindle structure, while the cytoplasmic substance itself is vacuolated and indistinctly stained (Fig. 9). In the cells of tissue cultures Lambert¹⁴ reported that scattered chromosomes of the dividing cell may fail to be incorporated into daughter nuclei, and he observed that dividing cells after reaching the stage of equatorial plate formation might show an arrest of the process and a subsequent break-up of the entire cell substance into small fragments. Cells in multiple mitosis have been most recently described and carefully studied in solid tissues by Levine,¹⁵ who has counted in them as many as 300 chromosomes. He suggests that the chromosomes may divide irrespective of nuclear organization or spindle formation and that, as indicated in the present examples, the process may be followed by disintegration of the chromatic material and possibly of the entire cell. He suggests that the liberated substances may have some influence in stimulating other cells to activity.

Possible answers to some of the many questions suggested by the findings in these fluids may come through study by the newer methods of supravital staining and tissue culture. Our recent attempts in these directions have not been successful, but the effort is being continued.

SUMMARY AND CONCLUSIONS

1. Neoplastic cells are readily demonstrable in pleural and peritoneal transudates resulting from neoplastic disease of the serosae. They are of interest not only because of their immediate value in clinical diagnosis but also because of the opportunity they afford for cytological study.

2. The tumor cells are believed to proliferate more or less actively within the fluid in which they are suspended. They occur as single cells or as clumps showing a more or less marked tendency toward organoid arrangement.

3. True tumor giant cells occur not infrequently and may be present in large numbers. While their formation probably depends upon unfavorable growth conditions the phenomenon may be for the individual cells a purposeful and protective mechanism.

4. These giant cells appear to result from successive fusions between individual tumor cells, with the gradual building up of great nuclear masses of varying morphology.

5. It is suggested that multipolar mitosis is a result rather than a cause of giant and multiple nucleation of tumor cells.

6. Multipolar mitosis is followed in some cases by cellular disintegration. No figures definitely identifiable as phases of nuclear reconstruction after multipolar mitosis have been seen in the present preparations.

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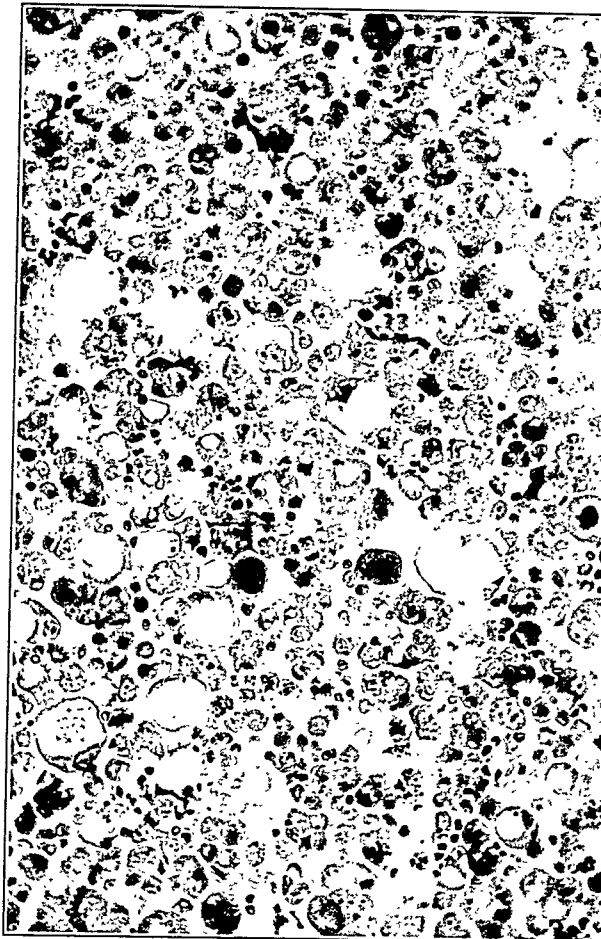
DESCRIPTION OF PLATES

PLATE 115

- FIG. 1. Vesicle formation in ascitic fluid. $\times 150$.
- FIG. 2. Single cells and cell clumps in ascitic fluid from a case of papillary cystic adenocarcinoma of the ovary implanted widely over the peritoneum. Near the right margin is a hyperchromatic cell in mitosis. Signet formations and cell inclusions are seen. $\times 150$.
- FIG. 3. A cell with a single, heavily chromatic giant nucleus and several sharply defined single nuclei, which show varying degrees of chromatolysis. At the top of the illustration is an irregular cell clump with two foci of cytoplasmic and nuclear fusion. $\times 500$.
- FIG. 4. At the margin of the (broken) circlet of large cells is a cell in multipolar mitosis. Toward the lower left is a disintegrating cell containing irregularly scattered chromosomes resulting from attempted multipolar division. In upper cell clump two diasters are seen and in the upper portion of the right hand clump two equatorial plates are slightly out of focus. $\times 300$.



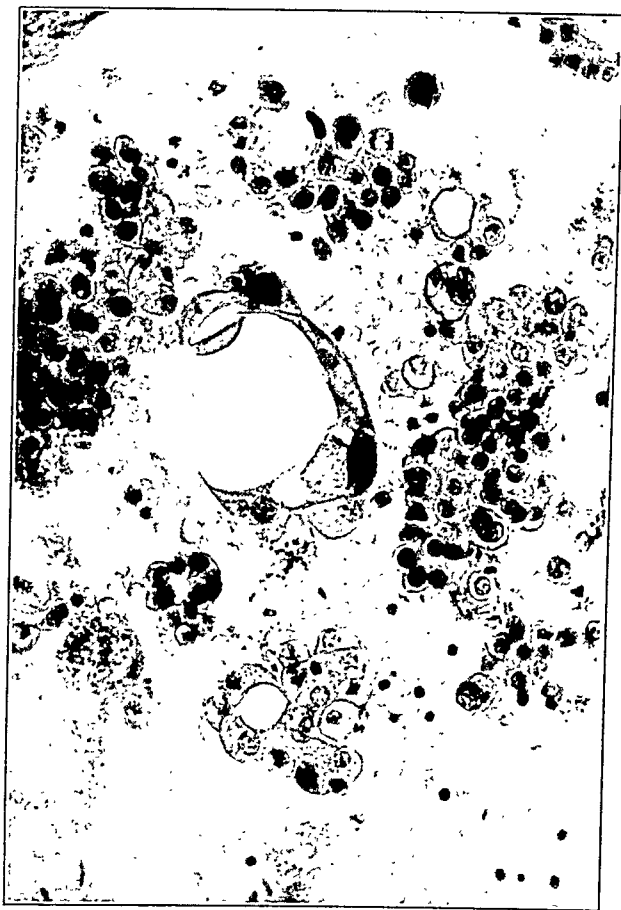
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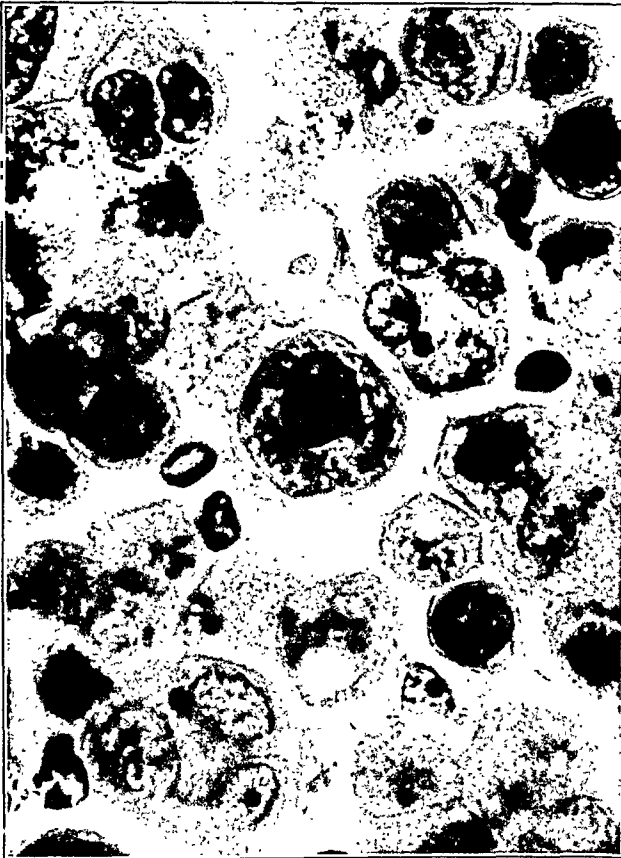
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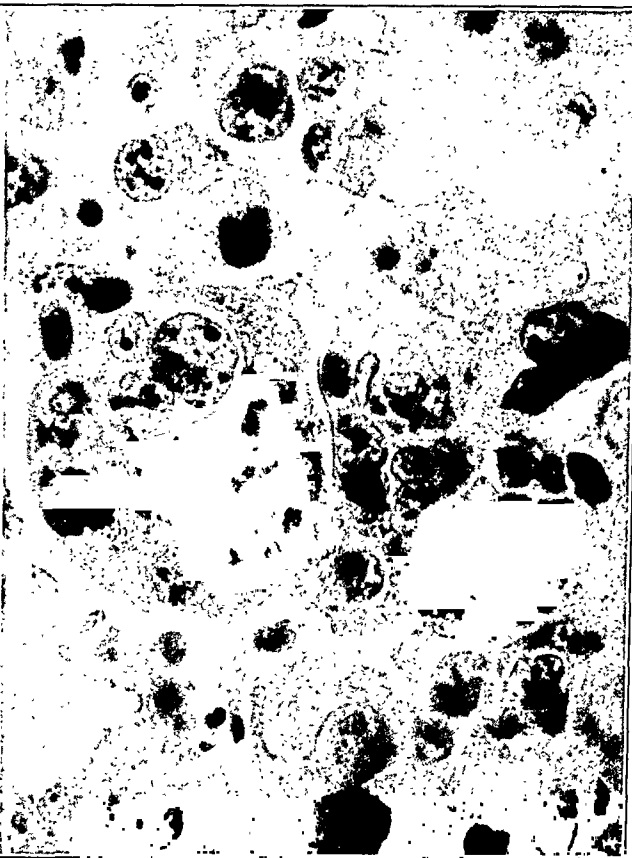
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PLATE 116

- FIG. 5. The central cell contains a large, deeply stained nucleus and three small nuclear bodies, the nuclear membranes of which are blurred and indistinct. The nucleoli are still demonstrable, although not evident at the optical plane shown. The nuclear membranes of one, and probably those of all the smaller masses, are in actual continuity with that of the central mass. The small bodies are believed to be elements in an original cell cluster whose cytoplasmic bodies have fused and whose chromatin content is concentrating in the central nucleus. $\times 750$.
- FIG. 6. Tumor giant cell enclosing a multinucleated cell. To the right a giant cell with circlet of nuclear lobules and scanty cytoplasm is seen. $\times 750$.
- FIG. 7. A cell clump with flattening and condensation of the marginal cells. The heavily stained nuclear mass is interpreted as an early phase in the growth of a giant nucleus. $\times 500$.
- FIG. 8. A smaller cell with nucleus of fusion type and heavy chromatin fragments in its marginal cytoplasm. $\times 750$.
- FIG. 9. A giant cell disintegrating after attempted multipolar division. The chromosomes are of unequal size, some of them barely visible in the photograph. A number of minute chromosomes have wandered into the cytoplasmic elongation at one pole of the cell. This cell can be followed through three sections, none of which shows any indication of spindle organization. $\times 500$.



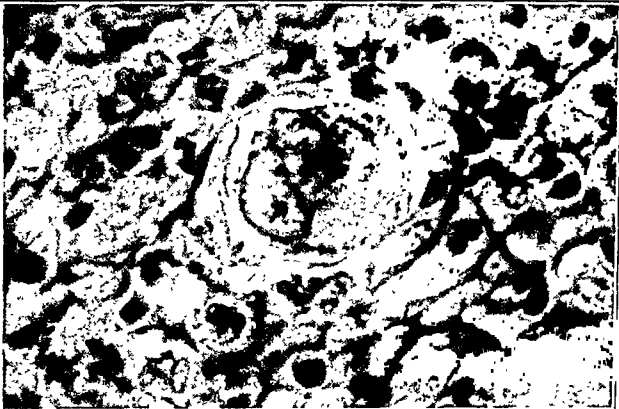
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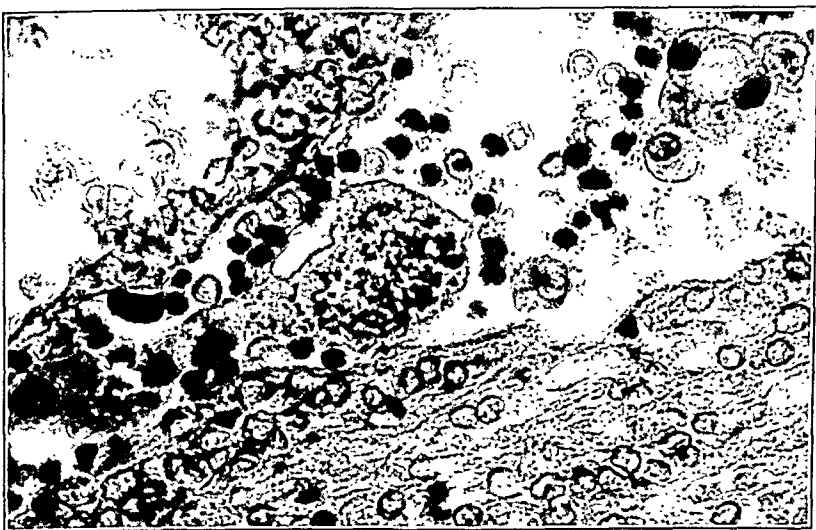
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THE PERSISTENCE OF TUBERCULOUS INFECTIONS *

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Some of the outstanding facts concerning tuberculosis, which must be appreciated by both the clinician and the pathologist, are its inherent insidiousness and its multiform manifestations. To assert positively that any lesion during life or after death is or is not of tuberculous origin often reveals in the final analysis definite and occasionally humiliating errors. There is no expert, no matter what experience or special training he may have had, who has not been "fooled" by this disease. Such mistakes may cover the entire subject in all its phases, for instance: (1) It is diagnosed as present when it is not. Sometimes this is true even with the finding of tuberculosis-like bacilli in the sputum, or the urine or feces. (2) The patient is assured that tuberculosis is not present when it is. Even macroscopic examination of excised tissues may fail to reveal its hidden presence. Orth¹ demonstrated this fact six years before Koch announced the discovery of the bacillus of tuberculosis, when he found, by the microscope, lesions in grossly normal lymph nodes of animals fed on fodder infected with tuberculous material. (3) The patient or his physician is told that the tuberculosis which he once had has been completely healed. Such a statement can never be made with any great degree of certainty and it is my purpose here to emphasize this fact.

There is nothing new in the idea that tuberculosis in the animal body may be hidden for indefinite periods; also that a tuberculous infection may remain latent or dormant for many years, being revealed only by increase of activity or postmortem examination. Soon after the positive identification of the Koch bacillus workers in laboratories began to demonstrate by animal inoculation the presence of virulent bacilli of tuberculosis in grossly normal lymph nodes removed from apparently non-tuberculous subjects. In 1890 Loomis² reported that in 8 of 30 such cases positive results were obtained by animal inoculation. These lymph nodes were not checked by microscopic examination. Pizzini³ found latent carriers of bacilli

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in 42 per cent of his cases. Similar reports were offered by Spengler⁴ and Straus.⁵ Kälble⁶ in 2 of 23 cases infected animals with lymph nodes apparently free from tuberculous lesions, even by microscopic examination. MacFadyen and MacConkey⁷ reported similar results. Numerous other students of human pathology have confirmed these experiments, notably Harbitz,⁸ and workers in the field of veterinary medicine have published many instances of concealed virulent bacilli of tuberculosis revealed by animal inoculation. Wang⁹ reviewed from the literature the total number of such examinations and found that in 357 instances grossly and microscopically normal lymph nodes gave positive results on inoculation into guinea pigs in 12 per cent of cases. In 1905 Weichselbaum and Bartel¹⁰ reviewed the subject and concluded that these organisms might remain latent without multiplication.

Many years before, Kurlow,¹¹ after experimenting with animals, had expressed the belief that wherever there is a caseous focus there remains for that individual the danger of a further spread of tuberculous autoinfection, and that lesions of tuberculosis can be regarded as healed only when the process shows an old scar or fully completed calcification. Lubarsch¹² tested these conclusions by grinding up partially calcified bronchial lymph nodes and inoculating guinea pigs with them; in a considerable percentage he recovered virulent bacilli of tuberculosis. Rabinowitsch¹³ reported results in 5 cases; 4 with fully calcified nodes, and all positive by guinea pig inoculation. Schmitz¹⁴ confirmed these experiments, using partially or wholly calcified nodules from 28 clinical cases, and obtained positive results by guinea pig inoculation in 13 of them. In 2 of these cases he found bacilli of tuberculosis by direct staining methods. Wegelin,¹⁵ by a special method using antiformin, and intensive search, found recognizable bacilli of tuberculosis in 7 of 13 cases in which nodules of tuberculosis were apparently completely healed.

In 1900 Naegeli¹⁶ published the results of his "fine-tooth-combing" at autopsy for evidences of tuberculous infection. As in a successive series of cases his zeal and thoroughness increased, so increased the percentage of positive findings, the four series revealing 75 per cent, 90 per cent, 97 per cent and lastly 98 per cent of adults as harboring gross or histological evidence of tuberculosis. His aphorism "Jeder Erwachsene ist tuberculös" seemed justified. In this study he designated one group of cases as "latent active tuber-

culosis." Of 217 cases in which death was not due to tuberculosis he found the latent active type in 74 (34.1 per cent). These conclusions were the result of anatomical observations and were not controlled by inoculation experiments. Naegeli makes no apology for this anatomical standard but holds that its value rests on the thoroughness and training of the observer.

Birch-Hirschfeld,¹⁷ the previous year, reported that among 826 autopsies performed on subjects who died from accident or acute disease 171 (20.7 per cent) revealed tuberculous lesions. Of these 105 (12.7 per cent) were judged to be healed; 31 (3.8 per cent) were active and well advanced and 35 (4.2 per cent) revealed mildly active or latent processes. As other workers began to publish their statistics it became at once apparent, as would be expected, that the number of latent or comparatively inactive tuberculous lesions increased with the age of the persons examined, just as did the percentage of those showing evidences of infection. Although the percentages of various authors differed over a considerable range the fact just stated continued to stand out in clear-cut prominence. Thus Lubarsch¹⁸ in 1913 found that in 139 bodies of tuberculous subjects between 1 and 16 years of age, only 33 (23.7 per cent) revealed calcification, although in none was the lesion completely healed, while at the age of 40 years, latent or healed lesions were more frequent than the active ones. He also emphasized how careful histological examinations may often reveal activity where grossly none is suspected. Reinhart,¹⁹ in 1917, found in 460 postmortem examinations no lesions in 28 newborn infants, active tuberculosis in 29.16 per cent of 72 children aged less than 16 years, and that of 360 adults 96.38 per cent had signs of the disease. In 63.9 per cent of this latter group the process was regarded as healed. Mönckeberg²⁰ examined the bodies of 85 soldiers who died in the World War. In 27 of these there were signs of tuberculosis. In Hart's²¹ series of 573 soldiers, 196 (34 per cent) had tuberculous lesions, of which 151 (26.8 per cent) were quiescent.

In this same year, 1917, Opie,²² using X-ray plates to identify the less readily distinguishable nodules in lungs and hilum nodes, discovered that whereas about 8.3 per cent of infants aged up to 2 years were tuberculous at death, in from 2 to 10 years this percentage increased to 44, from 10 to 18 years to 66.7 and beyond that period 100 per cent of bodies (50) revealed lesions of tuberculosis.

In a later study, 1927, Opie and Aronson²³ by guinea pig inoculation endeavored to ascertain what proportion of apparently healed lesions contained living bacilli of tuberculosis. Material from 169 bodies was thus examined and gave positive results in 52 cases (30 per cent). When they selected pulmonary tissues, which were apparently free from tuberculosis (although signs of the disease might be present in other portions), in 33 cases 15 (45+ per cent) gave positive results.

In spite of rather wide variations in percentages it is quite clear that almost every adult (at least in the past generation) had been at one time infected with virulent bacilli of tuberculosis, and that many of them at the time of death still harbored infective organisms, even though their lesions might have reached a quiescent stage. I have purposely omitted references to clinical reports on this subject. Various tuberculin tests, studies of heredity, and records of recurrences of the disease, only confirm what Fishberg²⁴ denominates as "the frightful tuberculization of humanity."

My own experience closely parallels that of other students of tuberculosis, and for many years my attention has been focused on those cases demonstrating the latent or dormant characteristics of the disease. This emphasis has seemed all the more justifiable, not only because of the relative frequency of such cases, but also because the recent apparent subsidence of the virulence of the tuberculosis pandemic has led to extravagant statements about its curability, thereby tending to foster a spirit of blind optimism, which the facts do not warrant. Thus Jaquero²⁵ stated that after one year devoted to a "clinical cure of pulmonary tuberculosis" and another twelve months to "confirming and consolidating this result . . . the patient will be in a condition to return to a normal life without any risk. . . . Once this period is past the healing can be considered as definitive."

In the study, the results of which I wish to report, consideration has been given to the data of family and clinical history, physical examination and postmortem examination, both gross and microscopic. No attempt was made to verify the presence of the bacillus of tuberculosis either by staining or animal inoculation. The final criteria for the diagnosis of active tuberculosis rested on histological evidences of activity on the part of the cells in the tuberculous area, such as foci of connective tissue proliferation, giant cells and agglom-

eration of lymphocytes. Such standards do not entirely eliminate the possibility of errors, particularly as the lesions of silicosis often simulate a chronic tuberculous inflammation, but such sins of commission are more than outweighed by the sins of omission. Undoubtedly more painstaking methods would reveal a much larger percentage of lesions containing viable, virulent bacilli (Figs. 1-5).

A few cases are presented in abstract to illustrate the data employed in the tabulation:

CASE 1. A woman, aged 58 years, died suddenly from coronary occlusion. At the age of 12 she had suffered from tuberculosis of the spine. During the intervening forty-six years there had never appeared any manifestations of tuberculosis. However, the lymph nodes at the roots of the lungs were the site of progressive chronic tuberculous disease marked by the presence of fresh tubercles, giant cells and necrosis.

CASE 2. A man, aged 24 years, was killed suddenly in an automobile accident. He was a farmer and had always been in good health. There was no family history of tuberculosis. His physical development and condition was almost perfect. In spite of these facts examination of several enlarged lymph nodes at the hilum of the lungs revealed chronic progressive, well advanced tuberculosis.

CASE 3. An elderly man, aged 82 years, who had never been ill, and had a son living and well, on clinical examination presented no evidence of tuberculosis. Death was due to coronary sclerosis and infarction of the myocardium. In the lung were found old tuberculous lesions which revealed evidences of histological activity.

CASE 4. A woman, aged 64 years, died from bronchopneumonia following an operation for trifacial neuralgia. There was no family or personal history or clinical evidence of tuberculosis. A son was living and well. At autopsy a healed lesion was found in the lungs and active tuberculous lymphadenitis in the aortic and hilum nodes.

It was such occurrences as these that have led me to review the entire series of autopsies performed at The Mayo Clinic over a period of six years (1926-1931 inclusive), in order to determine the relative incidence of the various classes of tuberculous processes. The results represent the "run" of a moderately efficient mill.

During these six years approximately 3306 postmortem examinations revealed an incidence of some form of tuberculous lesion in 2064 (62.43 per cent). Of this group in 89 cases tuberculosis was either a principal or contributing cause of death, and in 1725 cases the tissues examined contained apparently entirely healed tuberculous processes. In Table I are arranged according to decades those cases in which were found active lesions unrecognized clinically. They total 134 (4.05 per cent) of the total examinations.

As previously admitted more detailed examinations or animal inoculations undoubtedly would have materially raised this percentage. Many lesions were pronounced healed because clear evidences of histological activity were not present. Previous workers have

TABLE I

The Incidence of Active Tuberculosis (Unsuspected Clinically) Found at Postmortem Examination

Age in years	No. of examinations	Cases of tuberculosis	Per cent
1 to 9	136	4	2.94
10 to 19	134	9	6.71
20 to 29	219	15	6.84
30 to 39	396	15	3.78
40 to 49	598	19	3.17
50 to 59	783	35	4.47
60 to 69	726	29	3.99
70 to 79	267	4	1.49
80 to 89	43	4	9.30
90 to 99	4
Total	3306	134	4.05

demonstrated that a certain percentage of these lesions will reveal virulent organisms. Even so, the number is sufficiently impressive to reemphasize the extreme tenacity of the tuberculous infection.

CONCLUSIONS

From this and the other reported studies the following conclusions would appear justifiable:

1. Tuberculous infections may occur and pursue their entire course without demonstrable clinical phenomena, that is, without attracting attention of patient or physician to their presence.
2. Recognized tuberculous infections may subside and be re-

garded throughout remaining life as healed and still remain continuously active.

3. Apparently healed tuberculous lesions may become clinically active after varying intervals.

4. No form of physical examination can assure any individual that he or she does not harbor the menace of active tuberculous infection.

5. The safest rule for physicians and patients alike is to regard tuberculosis as possessing an ever present potentiality for becoming active. One can almost say: "Once infected, always infected."

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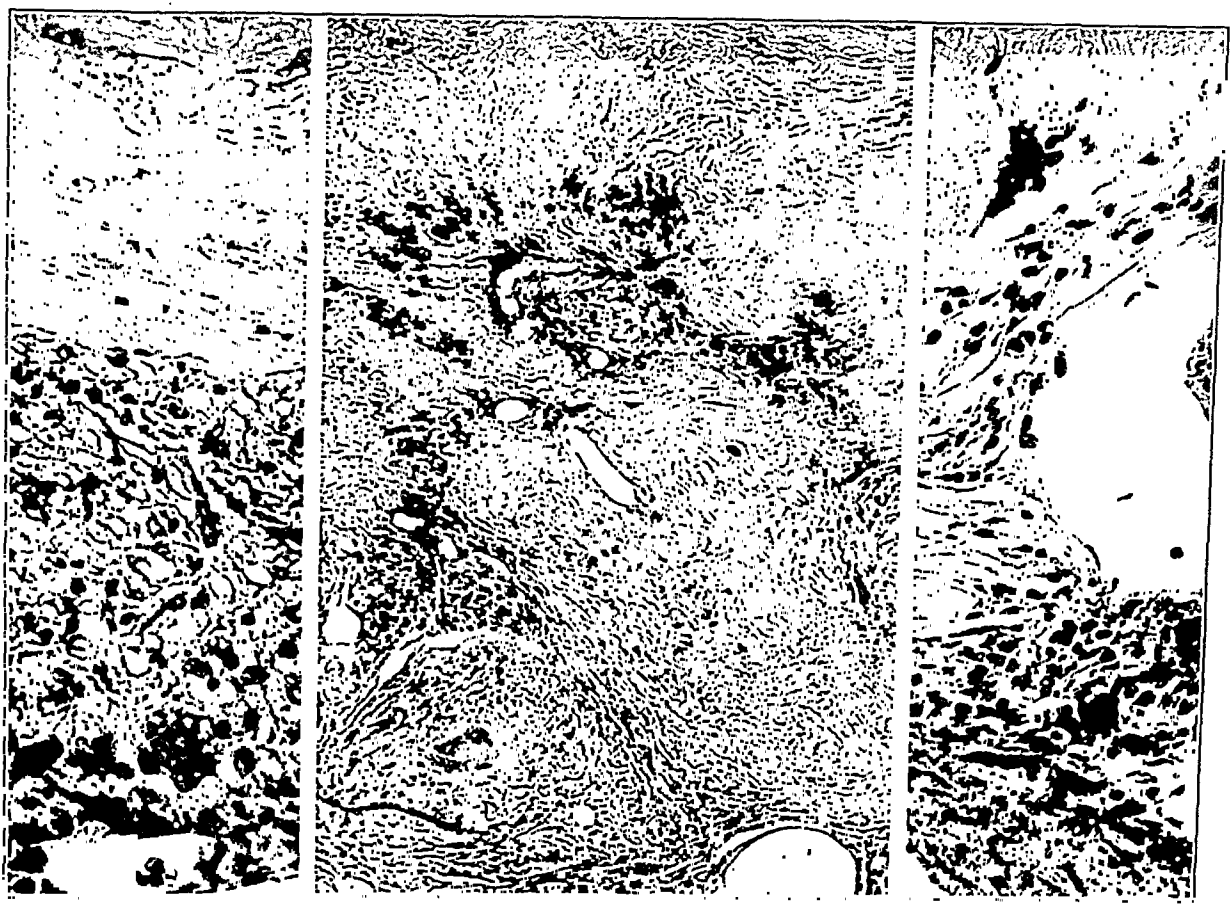
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DESCRIPTION OF PLATE

PLATE II7

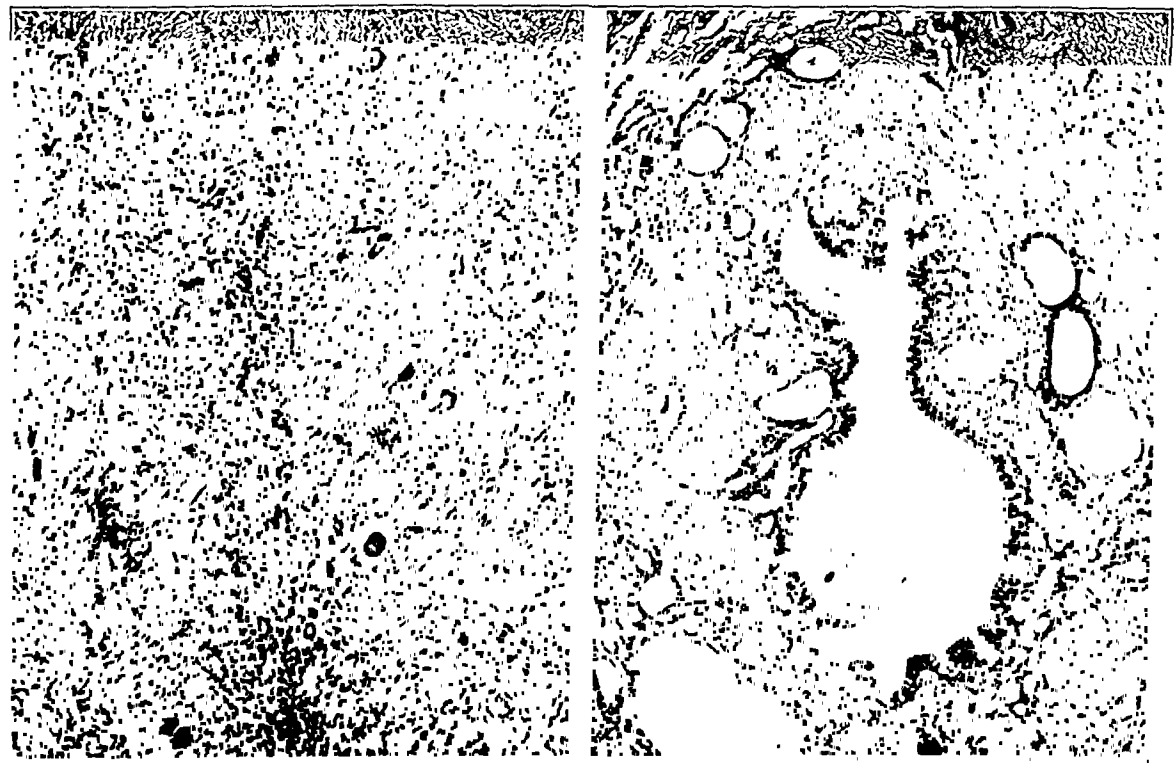
- FIG. 1. Hilum node from a man aged 74 years. Chronic tuberculosis. $\times 315$.
- FIG. 2. Hilum node from a man aged 60 years. Chronic tuberculosis. $\times 95$.
- FIG. 3. Lung from a man aged 69 years. Chronic tuberculosis. Minimal histological signs of activity. $\times 275$.
- FIG. 4. Lung; same case as that shown in Fig. 2. Active chronic tuberculosis. $\times 75$.
- FIG. 5. Bronchus; same case as that shown in Fig. 2. Chronic tuberculosis. $\times 50$.



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RHEUMATIC PERITONITIS *

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There are references to abdominal symptoms in "rheumatism" as long ago as 1635 when Ballonius,¹ in a discussion on the differential diagnosis between rheumatism and gout, referred to diarrhea, which occasionally accompanied rheumatism. He felt that this was beneficial, as the "humeurs" were, by this means, discharged from the body. At that time, and for a good while afterward, the term "rheumatism" was used in a general sense and was applied to such a large and varied group of diseases that one cannot be sure whether or not those to which Ballonius referred were rheumatic fever as we understand the disease today.

In 1752 Huxham,² in an article in which he discussed the localization of abdominal symptoms, referred to these as preceding rheumatic fever as we now know it. Since then there has been a sustained but fluctuating interest in these manifestations.

In recent times there has been not only a renewed and increasing interest in these symptoms, but some of these have been interpreted as resulting from rheumatic lesions in the peritoneal tissues. In an article on abdominal symptoms in acute rheumatic fever Giraldi,³ in 1930, discussed them under three headings: (1) the digestive group, (2) the pseudoappendiceal group, and (3) the peritoneal group.

There are several cases referred to⁴⁻⁷ in which acute abdominal symptoms developed during the course of, or were followed by, rheumatic fever. In some of the cases no lesion was found; in others a variety of conditions such as clear fluid, simple hyperemia with very little exudate, or fibrinous, purulent or even hemorrhagic exudate occurred. These have been interpreted as peritoneal reactions to the causative agent of rheumatic fever.

There is no unanimity in regard to the number of cases in which the involvement of the peritoneum is thought to be responsible for symptoms; in fact positive proof of peritoneal involvement is limited. This is probably due to the fact that the peritoneal lesions may

* Received for publication June 15, 1933.

not be visible to the naked eye and, more important than this, until comparatively recently rheumatic fever was looked upon as a disease that involved the heart and joints alone, and detailed microscopic study was largely limited to these organs.

Our conception of rheumatic fever today is much broader than it has been in the past. The disease is no longer thought to involve the heart and joints alone. We look upon it as a general infection that involves the body as a whole and results in a special kind of cellular reaction often with gross changes in various organs of the body. The cellular reaction in rheumatic fever is as specific for that disease as that found in tuberculosis. Like the microscopic lesion in tuberculosis that in rheumatic fever is not always the same in detail. The cellular reaction in tuberculosis is not that of the classical tubercle alone; it may be, and often is, much more diffuse. Neither does the cellular reaction of rheumatic fever result only in a typical Aschoff body; the lesions may be diffuse and yet be specific.

It has been recognized for a long time that there occurs a specific rheumatic lesion in the pericardium and in the pleura, and the signs and symptoms dependent on them can be recognized clinically. The peritoneum is, in general, similar to the pleura and pericardium and one might theoretically expect rheumatic lesions to occur there. If they occur, some of them should lead to clinical signs and symptoms.

In a comparatively large number of postmortem examinations on individuals dying of rheumatic fever at the Montreal General Hospital, but one has shown rheumatic lesions in the peritoneum. Following is a brief summary of this case.

REPORT OF CASE

Clinical History: A. B., male, aged 25 years, was admitted to the Montreal General Hospital with abdominal cramps and aching pains in the front of the chest, radiating to the back. Palpitation of the heart, dyspnea and a feeling of suffocation were present. There was a history of constipation, anorexia and loss of weight. The patient had had three attacks of rheumatic fever, one at the age of 4 years, one at 17 years and one in his 25th year. For some years he had had the symptoms resulting from an enlarged and incompetent heart. The winter before his admission to the hospital he had had his third attack of rheumatic fever, which had confined him to bed for a month. While in the hospital he had, in addition to the clinical signs and symptoms associated with an enlarged and incompetent heart, an abdomen that was rounded and a little distended, with abdominal muscles that were resistant. The liver and spleen were not palpable. There was slight, fixed dullness posteriorly. With rest in bed and proper medi-

cation most of the signs and symptoms partly or wholly disappeared. The abdomen, however, remained distended. The liver finally reached three fingers' breadth below the costal margin in the midclavicular line. The heart became more incompetent. During the last days of his life the patient was restless and complained of generalized abdominal pain. He died four months after admission to the hospital.

Clinical Diagnoses: Rheumatic pancarditis, chronic aortic valvular disease with marked insufficiency, mitral valve insufficiency, cardiac hypertrophy (*cor bovinum*), adhesive pericarditis, chronic passive congestion of the lungs, liver and kidneys, and rheumatic peritonitis.

POSTMORTEM EXAMINATION

The heart, which weighed 1150 gm., showed the changes that are found in recurrent rheumatic fever, and there was evidence of marked incompetence. The pericardium seemed to be completely obliterated by fibrous adhesions, in which a few discrete nodules 5 to 6 mm. in diameter were palpable. The myocardium contained a number of irregularly distributed, pale grayish foci 1 mm. in diameter. On all four valves a row of small, quite firmly attached vegetations were present, with least involvement of the pulmonary valve. In addition, old rheumatic lesions were present on the mitral and aortic valves. The pleurae were thickened and united in places by old adhesions. In some areas there were many petechiae on the parietal pleura. The lungs and spleen showed chronic passive congestion. The peritoneal cavity contained approximately 500 cc. of clear, straw-colored fluid. Anteriorly the parietal layer was smooth and glistening, while laterally and posteriorly there were plaque-like areas palpable in the subperitoneal tissues. The peritoneum in these regions showed the general characteristics of acute inflammation; it was dull, hyperemic and fine granules of fibrin were adherent. Similar lesions were seen in the peritoneum opposite the upper pole of the spleen. The walls of the intestines and the mesentery were edematous and congested.

MICROSCOPIC EXAMINATION

Heart: On microscopic examination the myocardium shows widespread areas of old, healed, rheumatic lesions. There are also numerous, typical, active Aschoff bodies, which are particularly abundant in the epicardium. One area in the endocardium of the left auricle shows an acute rheumatic lesion. The pale areas in the myocardium,

noted in the gross, prove to be rheumatic lesions. There is marked scarring and contraction of the mitral valve and the lesions of active rheumatic fever are present. Typical old and recent rheumatic lesions are found in the pericardium. In the connective tissue uniting the two layers of the pericardium there are numerous small spaces lined with mesothelial cells. The nodular areas seen in the gross are rheumatic lesions.

Aorta: In the first part of the aorta the periaortic tissues show the cellular changes characteristic of rheumatic fever. Sections of the parietal pleura, including the intercostal muscles and underlying fat, also contain foci of active rheumatic fever in the connective tissue.

Peritoneum: Blocks of tissue were removed from numerous places in the peritoneum. They were all cut so as to include the peritoneum and its underlying tissues. The great majority of these sections show only congestion of blood vessels, with marked edema and irregularly distributed monocytes. In other sections, including those taken from the lesions that were seen in gross, there are various stages of an acute inflammatory reaction. On the whole they are similar to the lesions noted in the heart. There are collections of monocytes, particularly about the blood vessels. Round and linear areas infiltrated with monocytes are also present. Among these monocytes there is an occasional lymphocyte, but no polymorphonuclear leukocytes are found. One of the sections taken from the peritoneal tissue includes a part of the psoas muscle. This section shows in the edematous, subperitoneal connective tissue and the psoas sheath a diffuse, acute inflammatory exudate, which occurs as linear bands of closely packed, elongated and round cells with deeply staining nuclei, and opaque, homogeneous, slightly acidophilic cytoplasm. Here and there are multinucleated cells. No necrosis is present. A few blood vessels show hyperplasia of the endothelial lining.

CONCLUSIONS

Except for the fact that the peritoneal lesions are diffuse, rather than focal, and sharply outlined, they are similar in every way to the so-called Aschoff bodies found in the heart in this case and in other cases of rheumatic fever.

We believe that these lesions are specific, resulting from the action upon the peritoneum of the causative agent of the disease.

It seems probable that the involvement of the peritoneum in rheumatic fever is more common than is generally thought. If careful microscopic examination is made of tissues from various regions of the peritoneum in instances of rheumatic fever, especially in the severe acute cases, the specific lesion may be found to be fairly common.

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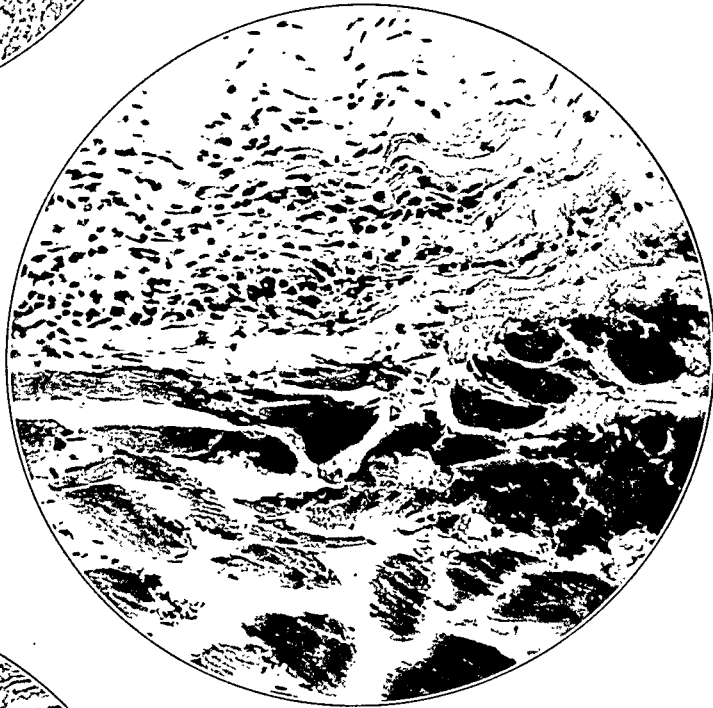
DESCRIPTION OF PLATE

PLATE 118

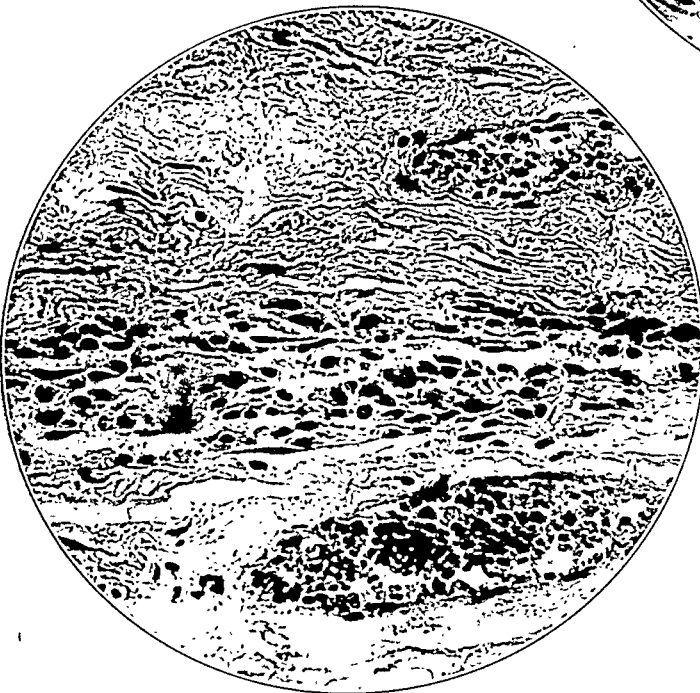
- FIG. 1. Section of heart showing old and recent lesions of rheumatic fever. Several Aschoff bodies are seen in the perivascular connective tissue.
- FIG. 2. Retroperitoneal lesion in rheumatic fever. Section through retroperitoneal tissue. The subperitoneal tissue shows edema and infiltration with monocytes. Part of the psoas muscle is seen.
- FIG. 3. Peritoneal lesions in rheumatic fever. The subperitoneal connective tissue shows dilatation of its blood vessels, edema and a linear area of cellular exudate. The cells are nearly all monocytes.



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Rhea

Rheumatic Peritonitis

NODULAR LESIONS OF THE PERITONEUM *

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Some years ago a nodule was sent to the laboratory for frozen section verification of the gross diagnosis of tuberculous peritonitis. The diagnosis of "foreign body granuloma encapsulating food particles" was returned at once. The history subsequently sent to the laboratory revealed the fact that the patient had had a previous operation for a perforated ulcer. This case, which is given in some detail later, served to fix my interest on the more unusual types of nodules of the peritoneum discovered at operation.

Most of the articles that discuss such nodules deal with isolated case reports of the respective conditions that may produce such lesions. Textbook descriptions are for the most part rather incomplete and sketchy. Kaufmann ¹ has a good chapter on lesions of the peritoneum and Hertzler ² deals with a number of peritoneal lesions under the name of "pseudotuberculosis." Since this subject as a whole has not received much attention in the literature I have undertaken the preparation of an outline of its possibilities and will use it as an index of the subject matter to be discussed. Our own cases are reviewed and they will be used, so far as possible, for illustration.

OUTLINE OF NODULAR LESIONS OF THE PERITONEUM AND OMENTUM

1. Tuberculosis.
2. Pseudotuberculosis (bacillary).
3. Syphilis.
4. Fungus infections.
5. Foreign body granulomas due to:
 - (a) Animal parasites.
 - (b) Extravasated blood.
 - (c) Gelatinous substances — including ruptured pseudo-mucinous cysts, mucocèles of the appendix and extraneous gelatinous substances.

* Received for publication April 1, 1933.

- (d) Oils and free fats — including extraneous lipoid substances, ruptured dermoid cysts and encapsulated foci of fat necrosis.
- (e) Extruded stomach and intestinal contents.
- (f) Extraneous foreign bodies, such as sutures, threads, bits of sponge, and so on.
- (g) Neoplasms, such as carcinomas, sarcomas, melanosarcomas, nodules from cases of Hodgkin's disease, and so on.

Since this discussion is to deal with the more unusual forms of peritoneal nodules I am omitting the subjects of tuberculosis and neoplastic nodules and will take up the other conditions in the order given.

PSEUDOTUBERCULOSIS (BACILLARY)

The term pseudotuberculosis has been applied by Hertzler and others to this whole group of lesions. There are two objections to this wider application: (1) the term was used by earlier writers in a specific sense to describe a group of bacillary infections which produced lesions resembling anatomical tubercles, and (2) the lesions are more commonly mistaken clinically for newgrowths, rather than for tuberculosis.

In its specific sense pseudotuberculosis includes a number of spontaneous infections which occur commonly in animals and occasionally in man. Among the organisms that have been reported as isolated from these conditions are *Bacillus pseudotuberculosis* of Pfeiffer, *Bacillus pseudotuberculosis rodentium*, *Bacillus pestis caviae*, *Bacillus abortus* (Bang), *Bacillus melitensis* and *Bacterium tularense*.

Tularemia is the only one of this group of infections that has occurred in our material. The sections from four human cases, including one complete autopsy performed in our laboratory, have been studied and the resemblance of the lesions to anatomical tubercles has been found to be striking. In the absence of the history it is doubtful if anything other than active tuberculosis in the stage of early caseation would have been suspected. Reimann and Rose³ recently reviewed a case of pseudotuberculosis due to a *Bacillus pestis* infection, reported some years ago by Roman, and suggested that it probably was tularemia. The authors mentioned the absence of giant cells as a differential point between tularemia and tuber-

culosis. The point is not to be relied upon, since there were numerous typical Langhans giant cells in many of the tularemic lesions of our series.

SYPHILIS

There are numerous statements that gummas of the peritoneum and omentum may occur, but I have found no descriptions of such lesions. In almost twenty years the diagnosis of syphilis of the omentum occurs only once in our files. On account of its rarity I am citing the case.

A patient admitted for acute cholecystitis was found at operation to have several white nodules in the liver. The omentum appeared flecked with pinkish white nodules.

Microscopically the material from the liver was definitely gummatous. The omentum was traversed by thick-walled vessels about which there were wide collections of lymphocytes and eosinophiles. Between the vessels the omentum was clear, save at one point where a nodule of ordinary granulation tissue was present. Near two of the vessels were minute foci of necrosis, one of which was circumscribed and contained numerous foam cells. The other lesion was diffuse, poorly defined and surrounded by a mononuclear and lymphocytic exudate. With the exception of the localization of the process about the vessels there was nothing characteristic of syphilis and the diagnosis depended upon the presence of the gummas in the liver and on a positive Wassermann reaction.

FUNGUS INFECTIONS

Primary fungus infections of the peritoneum appear to be rare. Actinomycosis¹ (see page 854) secondary to other lesions due to this organism has been described. Our experience has been limited to animal experiments. Monilia and Trichophyta injected experimentally have produced typical pseudotubercles in the omentum of guinea pigs and rabbits. Such tubercles showed little tendency to undergo caseation necrosis, though they regularly contained typical Langhans giant cells. *Aspergillus fumigatus* cultures from a human source, with which we have been experimenting for some months, sometimes produce pseudotubercles and at other times form abscesses closely resembling actinomycosis, and can be differ-

entiated from the latter only by oil immersion examination of the stained organisms. From our experiments it seems that almost any fungus pathogenic for man is capable of forming secondary lesions in the omentum which may grossly resemble tuberculosis.

FOREIGN BODY GRANULOMAS

By far the largest number of our cases of peritoneal nodules were foreign body granulomas. Although we have not had examples of the items outlined we have drawn on the literature in order to make the topical discussion complete.

(a) *Foreign Body Granulomas Due to Animal Parasites*: In some parts of the world parasites are common causes of nodular lesions of the peritoneum and several cases have been reported from this country. *Entameba histolytica* (Gunn and Howard ⁴) may cause granulomas of the bowel resembling carcinoma, with extensions to the peritoneum leading to fibrous encapsulation and foreign body giant cell formation. *Oxyuris vermicularis* (Goodale and Krischner ⁵) may become encysted in the mesentery and resemble tubercles. *Ascaris ova* ¹ and bits of chitinous membrane from echinococcus cysts (Del Campo ⁶) may become free in the peritoneal cavity and act as centers for foreign body granulomas. Hutchison ⁷ described peritoneal granulomas fourteen times and tubercle-like granulomas of the epiploic appendages twice in sixty-five cases of *Bilharzia* infestation reported by him from Tanta, Egypt. Several years before this report he sent me some *Bilharzia* material and I studied the granulomas at first hand. It was the best material for the study of foreign body giant cell formation I have observed. The ova had worked their way into the stroma of the fat tissue and set up inflammatory processes. Many embryos had died and leukocytes had entered the shells and digested the embryonal debris. Mononuclear cells were gathered about the outer surfaces of empty eggs, had become granular and fused into large giant cells. The ova shells themselves acted as small bottles or flasks into which the mononuclear phagocytes were seen to be passing in considerable numbers. At one end of such a flask occasionally might be seen separate and distinct phagocytes, while at the other end were large, fused, syncytial masses filling that part of the cavity. The indications from the study of the *Bilharzia* material pointed toward fusion as the mode of formation of giant

cells, there being no mitoses and no evidences of direct multiplication of the nuclei of phagocytes.

(b) *Foreign Body Granulomas about Extravasated Blood:* The intra-peritoneal hemorrhages that appear most likely to produce foreign body granulomas are those that follow the rupture of graafian follicles, corpora lutea and tubal pregnancies, or the repeated, small, bloody extravasations that sometimes accompany the backward flow of menstrual blood through the tubes in instances of atresia of the cervix. Sampson ⁸ has pointed out that bits of displaced endometrium may exude blood during menstrual periods.

In our series there were six cases in which the tubes, broad ligaments, dependent peritoneal surfaces and mesoappendices were covered with small, slightly raised, yellowish brown nodules. The pathogenesis of the lesions was not entirely clear. They occurred five times in association with long standing tubal infections, two of which were diagnosed tuberculosis at operation, and once in a patient who had had a spontaneous abortion three weeks before a laparotomy was performed. A tubal abortion of a twin pregnancy was considered before operation, though no evidences of it were found when the abdomen was opened and no explanation of the multiple hemorrhagic spots was obtained.

Microscopically the nodules in all of the cases were similar and consisted of masses of phagocytes filled with yellowish brown pigment. Among these phagocytes were multinucleated giant cells grouped about crystalline deposits. Careful search was made in each instance for endometrial transplants but none was found. The nodules in this series were light yellowish to pale brown in color. Several textbooks of pathology mention the fact that blood spots in the abdomen may take up sulphuretted hydrogen (pseudomelanosis), which von Gierke ⁹ states changes them to a grayish green or black color.

(c) *Foreign Body Granulomas about Gelatinous Substances:* That gelatinous substances freed through the rupture of pseudomucinous cysts of the ovaries and of mucocèles of the appendix may produce foreign body granulomas of the peritoneum is well known. In addition to examples of these we have had a case of a mucocèle of Meckel's diverticulum with peritoneal extensions, and one of multiple granulomas in a young woman in whom the source of the material was not discovered.

I have reviewed our records of eighty-two large ovarian cysts, exclusive of dermoids, and have found only six of these which at operation showed peritoneal extensions or nodules. Three of them were malignant and the sections both from the cysts and from the nodules were carcinomatous. Another cyst appeared to be a ruptured simple pseudomucinous cyst, the transplants of which contained secreting epithelium producing much gelatinous material. This lesion appeared both clinically and pathologically to be the condition generally diagnosed as *pseudomyxoma peritonei*. The patient made a complete recovery and was entirely well when last interviewed five and one-half years after the operation. Two other cases showed simple pseudomucinous cysts and the extensions from them were essentially foreign body granulomas, though there were small islands of epithelium with colloid in one. The peculiar finding in these nodules was the unusually large amount of fibrous connective tissue produced, in comparison to the small amounts of colloid, as well as the small size of the giant cells.

We had one instance of a ruptured mucocele of the appendix though the extrusion of the gelatinous material appeared to have been too recent to permit granuloma formation. A patient had been operated upon for a ruptured appendix which was found to be walled off in the ileocecal fossa. The appendix was not removed, a drain was inserted and recovery followed eventually. Six months later the patient was readmitted with nephrolithiasis. A nephrectomy was attempted but owing to the poor condition of the patient it was abandoned. Two days later he died. At autopsy several large kidney stones were found. The omentum was adherent in the ileocecal fossa and beneath it was a yellowish green-colored gelatinous material, which bathed the cecum and stained the surrounding peritoneal surfaces. The material was exuding from a small dilated sacculle, the wall of which was thinned out and proved to be the remnant of the old appendix. A double handful of the mucoid material had gravitated to the pelvis where it was lying free behind the bladder.

In reviewing a series of cases of *pseudomyxoma peritonei* associated with appendiceal mucoceles, and in reporting a case of his own, Dieker¹⁰ made the following points. Mucocele of the appendix follows obliteration of the lumen, usually in the proximal half. This is followed by the continued secretion of the appendiceal mucosa in the

encysted distal portion. As the process continues the muscularis becomes stretched to such an extent that the fibers may become separated and the contents escape from small openings formed without actual rupture. As the lumen is no longer open, contamination by feces from the intestinal canal no longer occurs and the enclosed pseudomucin becomes sterile and acts only as a chemical irritant. The gelatinous secretion has at least one fraction which is insoluble in body fluids and this becomes organized with foreign body encapsulation.

If epithelial transplants occur at the time of the escape of the gelatinous material the foci of pseudomucin production may become widespread and the condition known as *pseudomyxoma peritonei* result. Frank ¹¹ cites an instance of this disease following the rupture of a pseudomucinous cyst of the ovary in which 350 pounds of gelatinous material was removed from a patient in twelve operations.

During an exploratory operation on a patient with a large abdominal mass lying to the left of the umbilicus a colloid cyst of Meckel's diverticulum, about 8 cm. in diameter, was found. At the junction of the diverticulum and the intestine there was a firm leiomyoma about 5 cm. in diameter. The omentum, mesentery, and neighboring peritoneum were covered with thin-walled cysts. A clinical diagnosis of colloid carcinoma was made. The pathological examination revealed a mucocele of Meckel's diverticulum without evidence of malignancy or rupture, a benign leiomyoma and foreign body extension cysts of the peritoneum filled with pseudomucin.

The material for the final example of foreign body granulomatosis in this group was from a young married woman, 26 years of age, who was operated upon for what appeared to be an attack of acute appendicitis, but which proved to be an appendix of the interval type. At operation the mesoappendix, broad ligaments and pelvic peritoneum were covered with small clear nodules containing a non-fatty gelatinous material of undetermined source. Sections showed typical foreign body granulomas with numerous large giant cells. The gelatinous material contained many concretions suggesting starch granules. The possibility of a gelatinous contraceptive as the source of this material was suggested. This seemed possible, as Dr. Mortimer Cohen recently had a case* of multiple granulomas

* This case was presented at a meeting of the Pittsburgh Clinical-Pathological Society.

of the tubes following lipiodol injections for roentgen-ray examination. The use of such substances was denied by the patient and the lesions remained unexplained. However, the possibility of gelatinous contraceptives as a source of granulomas seems practicable.

(d) *Foreign Body Granulomas about Oils and Free Fats*: There are at least three kinds of oily substances that may give rise to foreign body granulomas in the peritoneal cavity. These include extraneous oils and fatty compounds introduced during operation, sebaceous material from ruptured dermoid cysts, and the later stages of fat necrosis associated with diseases of the pancreas.

At one time it was a fairly common practice to introduce oily substances into the abdomen to prevent peritonitis and adhesions. Wilkie ¹² in 1910, at which time this procedure was being widely considered, published an article discussing the relative merits of olive oil, vaseline, mucilage, gum arabic, paraffin, lanolin, gelatin-agar and fibrolysin for the prevention of adhesions and peritonitis. He recommended vaseline smeared over the intestines as the best of the group because it did not break down into irritating fatty acids and it retarded the absorption of bacteria. Such practices were soon found to produce, rather than prevent, adhesions and were abandoned.

A case report and review of several cases of ruptured dermoid cysts by Herzog ¹³ throws light on the fate of free fats in the peritoneal sac. Herzog's case was that of a 68 year old woman who had had a large dermoid cyst ruptured in an accident fifteen days before death. At autopsy the peritoneum was covered with nodules and there were also 800 gm. of free fat, sebaceous material and hair in the cavity. Some of the fat appeared to have separated into free droplets and fatty acids and to have become fixed to the walls by fibrin. This material was quickly overgrown by the mesothelium and became organized through the ingrowth of fibroblasts and the formation of foreign body giant cells and capillaries. Some of the granulomas were found deeply embedded in the subperitoneal tissues. Among the other cases cited by Herzog was one of Fränkel's, in which the squamous epithelium of a ruptured dermoid cyst coated the peritoneum with an epithelial layer resembling that of the skin.

The appearance of early fat necrosis in the peritoneum and omentum is now widely recognized. It does not seem to be well understood that foci of fat necrosis sometimes persist for weeks and give

rise to typical granulomas difficult to differentiate microscopically from those formed about extraneous fats. In his experimental studies Wells¹⁴ followed the development of fat necrosis after the injection of pancreatic juice. He concluded that both trypsin and lipase were necessary, trypsin to produce the necrosis and lipase to act in breaking down the fats. He further determined that bile salts may produce necrosis but do not digest fats. Free fat droplets and soaps so liberated were not toxic, though they often took up calcium and became insoluble. Experimental lesions disappeared after eleven days. Distant lesions seemed to be due to emboli of pancreatic cells. Rewbridge¹⁵ found that injected bile salts produced a special form of fat necrosis in normal dogs, but caused hemorrhages without necrosis in depancreatized dogs. He concluded that after the escape of bile salts, fat necrosis may occur independently of pancreatic disease though not independently of pancreatic juice. The observation is of especial interest in explaining several of our cases of fat necrosis associated with long-standing gall-bladder disease.

In our series there were eight cases of foreign body granulomatosis of the peritoneum due to fatty substances. Three were interpreted from the findings as being due to extraneous oily substances.

In the first of these cases the patient had had two previous operations, one of which was a gastro-enterostomy performed several years before. On opening the abdomen there were extensive adhesions between the coils of intestines, with numerous cyst-like bodies attached closely or by short pedicles over the sigmoid and uterus. There were numerous cyst-like inclusions in the omentum filled with an oily substance soluble in alcohol and chloroform. A clinical diagnosis of granulomatosis about oil used at a previous operation was made.

Microscopically the cyst-like bodies resembled normal fat with extensive infiltration between the globules. Closer observation showed them to be made up of closely packed, clear, globular spaces surrounded by giant cells. Between the globules there was a fibroblastic granulation tissue infiltrated with mononuclears, lymphocytes and eosinophiles, particularly marked about the vessels. There were large areas packed with masses of multinucleated cells containing clefts and irregular cysts.

The second case classed as due to extraneous fats was discovered during the repair of an incisional hernia. The patient had been oper-

ated upon three times between 1912 and 1915 for a supposed ulcer of the duodenum. The surgeon stated that there were so many adhesions he could not tell what had been done, though he assumed that oil had been used at some previous time.

The third case occurred about a walled-off area in the ileocecal fossa following the repeated packing of a sinus with bismuth paste. Both the tract and the adhesions about the cecum were filled with dark nodules so that a secondary melanotic sarcoma was suspected.

Microscopic examination showed globular spaces, for the most part empty, though a few were lined with black pigment and surrounded by large, multinucleated giant cells. Other types of exudative cells were almost entirely absent in this lesion.

Of the five cases associated with pancreatic and gall-bladder disease one is of interest because the date of onset was known definitely. This patient, aged 7 years, was struck by an automobile and suffered several fractures, as well as internal injuries. The abdomen began a gradual and continued enlargement and an exploratory operation was performed forty days after the accident. An encysted peritonitis containing 500 cc. of fluid was found in the lesser peritoneal sac. The peritoneum and omentum appeared inflamed and were studded with small, discrete, yellowish white tubercles. A diagnosis of tuberculous peritonitis was made.

Microscopic examination proved the nodules to be foci of fat necrosis which were practically all encapsulated and in which there were a fair number of giant cells. The patient recovered.

In another case in this group there was a history of a recurrent attack of abdominal pain preceding death. At autopsy the findings included acute hemorrhagic pancreatitis, fresh foci of fat necrosis in the peritoneum and completely organized granulomatous nodules about areas of fat necrosis. The latter were probably accounted for by the earlier attack.

Of the three remaining cases of encapsulated areas of fat necrosis, in one case this condition was associated with carcinoma of the pancreas, in the second with repeated attacks of cholecystitis, and in the third with cholelithiasis. In this last case the surgeon found the head of the pancreas enlarged and unusually firm. In the latter two cases free fat droplets, organization and giant cell formation were present. Actual necrosis was absent (Fig. 1).

(e) Foreign Body Granulomas about Extruded Stomach and Intestinal

Contents: When an ulcer of the stomach ruptures and the stomach contents escape into the peritoneal cavity a fatal peritonitis generally ensues. Occasionally enough of the material is removed at an emergency operation to permit recovery. Again the peritoneum is able to take care of the bacterial contamination, and food particles are left to produce foreign body reactions. Occasionally there is a case reported where vegetable matter has been found in the peritoneum, without a history of previous operation or a definite history of an acute abdominal attack. Shennan¹⁶ reported such a case and identified the vegetable particles as encapsulated oat seeds. In newly born infants foreign body granulomas have been described about extruded meconium (Boikan¹⁷). We have not found any instances of meconium peritonitis of this type, though we have had four cases of foreign body granulomas about vegetable material in our series. The first of these was mentioned in the introductory paragraph. A brief summary of this case follows.

The patient was first admitted with a duodenal perforation of about four hours duration. At operation a large calloused ulcer was found. The abdomen contained considerable fluid and stomach contents, in which the coverings of peas were recognized. The perforation was closed and a gastro-enterostomy performed. The patient recovered and remained well for six months when he began to have discomfort in the region of the stomach and retention of gastric contents. Ten months after the first operation he was readmitted to the hospital. At operation the omentum was found to be rolled up and adherent to the colon and to the abdominal scar. In the right upper quadrant there was a mass of adhesions which were broken with difficulty. All through the omentum and adherent to the colon were small, white, lymph node-like nodules varying from a pin-point to 0.5 cm. in diameter. A clinical diagnosis of tuberculous peritonitis was made and a piece of tissue sent to the laboratory for a frozen section. This was found to be a foreign body granuloma formed about vegetable matter. Further study revealed occasional areas that contained bits of striped muscle. Figure 2 shows a nodule from this patient.

Following this case a series of animals was injected with stomach contents. When untreated contents were used the animals died of peritonitis. When cooked sterile contents were used the lesions found were reproductions of the granulomas seen in the case men-

tioned above. Cooked muscle tissue injected into guinea pigs intraperitoneally was found to persist still undigested and without loss of striae when the animals were killed ten weeks later.

In the second case the patient was an Italian laborer, 52 years of age, who had been injured in a fall some months previous to admission. He was admitted to the hospital for the repair of inguinal and ventral hernias. He stated that he had not been sick a day in his life. When the abdomen was opened the peritoneum, omentum and intestinal serosa were thickly studded with nodules resembling tubercles. A diagnosis of tuberculous peritonitis was made and specimens sent to the laboratory. They were much like the granulomas in the first case, though their origin was unknown.

The third case was discovered at operation when a loop of bowel which had ruptured six weeks before and which had been pulled up and sutured outside the abdomen was being returned to the peritoneal cavity. Again a diagnosis of tuberculous peritonitis was erroneously made.

The fourth case followed the rupture of a stomach ulcer nine months earlier. At autopsy this proved to be a carcinoma of the stomach. The peritoneum and omentum were studded with nodules, which on microscopic examination proved to be secondary growths. In two of them, however, there were found encapsulated vegetable remains.

(f) *Foreign Body Granulomas about Sutures:* There have been a great many small localized granulomas about sutures in our material, but they have been incidental findings and have caused no difficulty in diagnosis.

SUMMARY

A series of nearly thirty cases of nodular lesions of the peritoneum, which were of interest from the standpoint of surgical diagnosis, have been collected from our material and an outline has been prepared to facilitate the more rapid classification of such lesions.

The writer begs to express his most sincere thanks to the various members of the surgical and gynecological staffs of the Allegheny General Hospital for permission to cite their cases.

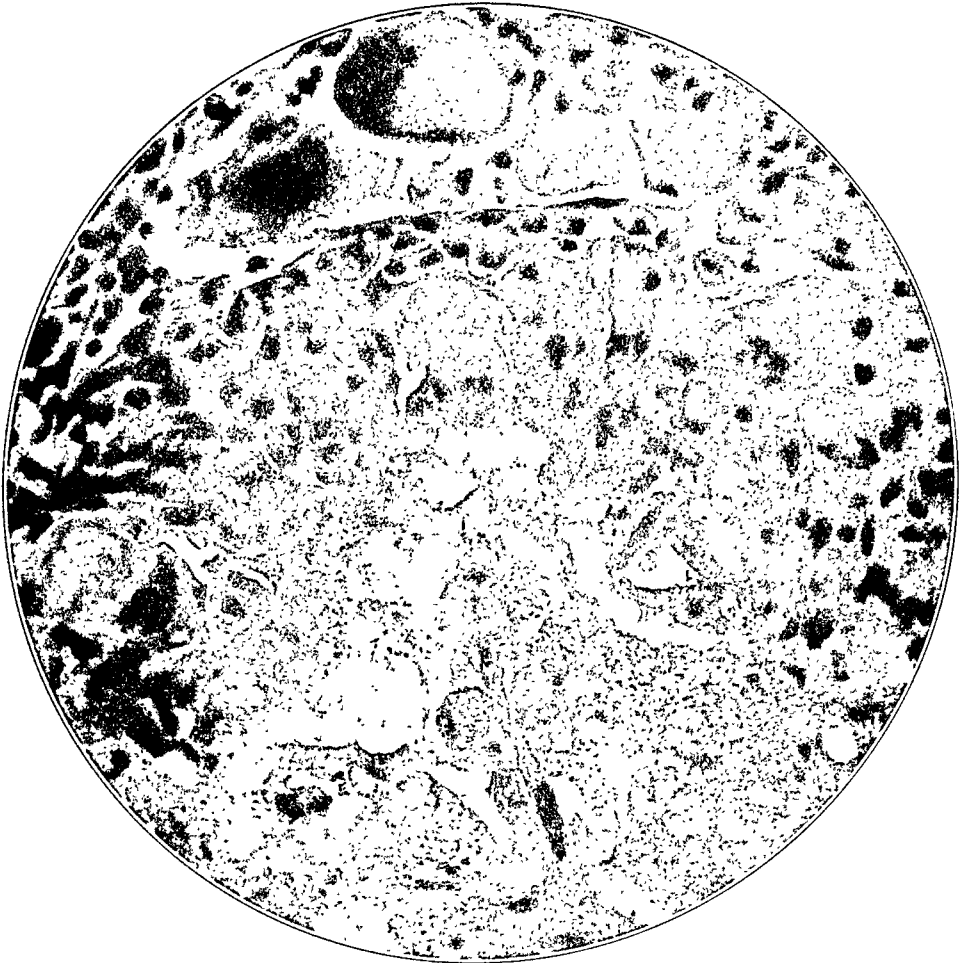
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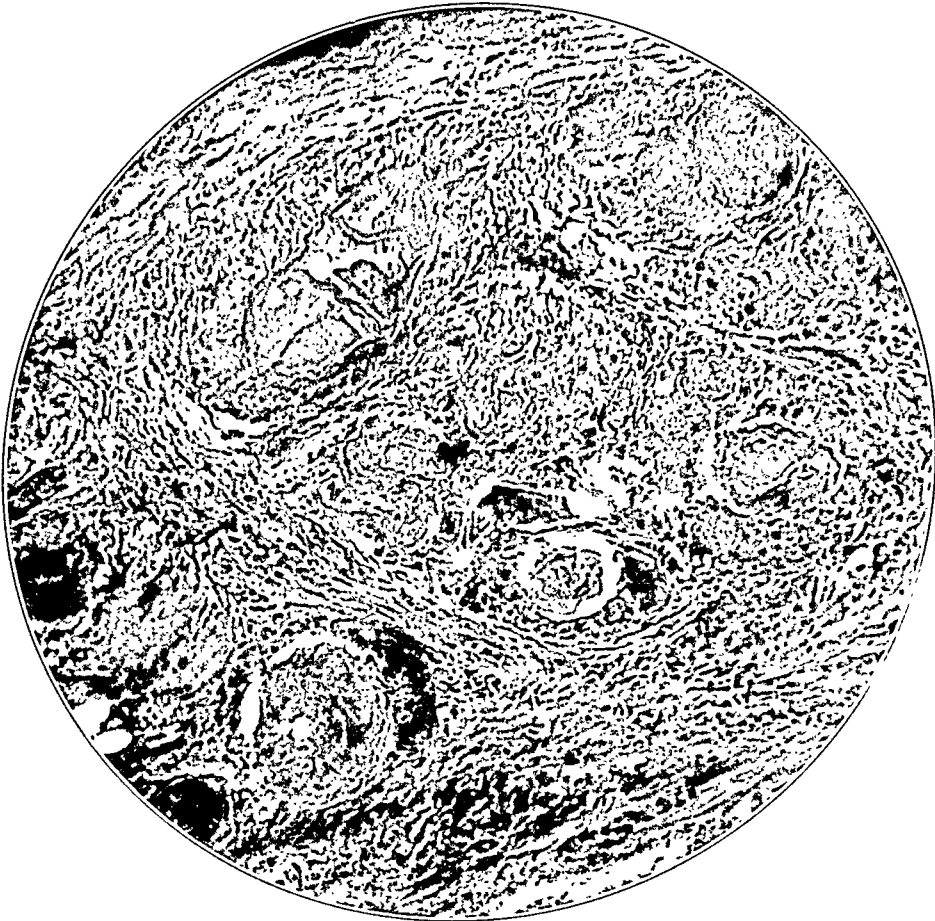
DESCRIPTION OF PLATE

PLATE 119

- FIG. 1. Foreign body granuloma of the mesentery due to the organization of fat necrosis in a case of chronic cholecystitis and cholelithiasis without rupture. $\times 150$.
- FIG. 2. Foreign body granuloma of the peritoneum due to the extrusion of vegetable material through a ruptured duodenal ulcer. This lesion was of ten months duration. The condition was diagnosed tuberculosis on inspection of the peritoneum at the second operation. $\times 30$.



1



2

GROWTH INHIBITOR IN KIDNEY DESICCATES *

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As early as 1913 Carrel¹ determined that fibroblasts and epithelial cells multiply indefinitely in a medium of plasma and embryonic juice. In 1926 Baker and Carrel² found that the agent responsible for the multiplication was contained in the protein fraction of the embryonic juice, and later the same year proved that proteoses prepared from many different proteins stimulated the proliferation of fibroblasts and other cells.³ Their explanation of the stimulating effect of embryonic juice was that the enzymes contained in it hydrolyzed the protein to proteose. Peptones, peptids and amino acids resulting from further hydrolysis contributed to the nutrition of the fibroblasts.⁴ These experiments leave little doubt as to the power of certain primary protein derivatives to excite karyokinetic activity in cells removed from the body and placed *in vitro* under conditions favorable for growth. Within the body of an animal the cell is in an environment made so complex by chemical, nutritional and nervous influences that a test of the action of substances on mitotic activity is not easy to make. Carrel⁵ found that the application of dog subcutaneous tissue to skin wounds of dogs delayed cicatrization. The author⁶ observed some stimulating effect of macerated epidermis, especially that of fetal origin, on the regeneration of epidermis in cutaneous wounds. Dvorak and Byram⁷ applied various macerated tissues such as liver, kidney and spleen to wounds of the skin, but found no striking acceleration of healing.

Physiological processes on analysis often are found to consist of two components which operate in opposition to each other. Some biologists have expressed the view that the mechanism of tissue growth consists of a balance and imbalance of inhibitory factors and formative stimuli. Evidence of the presence *in vitro* of definite growth stimuli is satisfactory. Proof of their existence within the animal is less certain. When inhibition within the body is considered the situation is very different. Several inhibitors are known as defi-

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nite substances with specific properties. Up to now most but not all of the investigations along this line have been with organs producing hormones. Halsted⁸ successfully transplanted parathyroid only when one-half or more of the parathyroid tissue had been removed. Loeb⁹ found active thyroid regeneration only after the operative removal of about three-fourths of the thyroid gland and, of even greater significance, he observed an inhibition of regeneration in partially thyroidectomized animals that were placed on thyroid feeding.

We began our study of the operation of inhibitors of cell proliferation with observations on the pancreatic islets of suckling rats injected with insulin.¹⁰ The results were decisive. Up to 20 days of age mitoses were abundant in the pancreatic islets of rats. Iletin injected intraperitoneally reduced the mitotic rate from one-third to one-tenth that of the normal, with no adverse action on the general nutrition of the animal. In fact growth, as shown by the total body weight, was in several instances accelerated. The effect of parathyroid hormone on the proliferative activity of the parathyroid glands was determined.¹¹ In order to estimate the hormonal effect by a determination of the hypercalcemia we had to employ half-grown rats, and the rate of parathyroid proliferation in these was such that an accurate enumeration of all the mitoses in the entire glands was necessary. Formalin-fixed tissue proved to be entirely unsatisfactory for this purpose. A particular chrome-formol fixation gave a clear definition of the karyokinetic figures. Maximum, minimum and average rates of proliferation were then established in a series of normal rats of the same age as those used in the hormone injections. The injections of two different preparations of parathyroid hormone reduced the proliferative rate to a fraction of the normal. This reduction was accomplished not only by the hypercalcemic dosage but by relatively small doses which produced no demonstrable change in serum calcium. The reduction in mitotic rate was found to be a more delicate test for hormonal activity than either hypercalcemia or the occurrence of toxic lesions in parenchymal organs. We made several quite instructive tests with an inactivated hormone prepared by Tweedy and Torigoe.¹² By a chemical change in the hormone its hypercalcemic action is removed (inactivation), but the process is reversible and its property of producing a hypercalcemia may be restored (reactivation). The inacti-

vated hormone, perfectly inert as far as blood calcium and toxicity are concerned, was equally as effective as the active hormone in its inhibiting action on the mitotic rate of the parathyroid gland.

Microchemical tests of the effect of epinephrin on the adrenal medulla were made by Ogata and Ogata¹³ who, using toluidin blue as a histological reagent, observed that the depth of green in the medullary cells was proportional to the amount of epinephrin or preëpinephrin substance present. They found only a trace of green after adrenalin administration. Samson¹⁴ confirmed these findings by demonstrating reduced amounts of epinephrin content in the medullary cells in dogs receiving intravenous epinephrin. Further confirmation of a diminished content of secretion within the gland following dosage with adrenalin was obtained by Cramer,¹⁵ who observed that the cells of the adrenal medulla lose their chromaffin staining after injections of epinephrin. He concluded from this that the adrenal gland has the power to control its functional activity in such a way as to prevent the gland from stimulating itself to complete exhaustion. Employing suckling rats we established the normal proliferative rate of the adrenal medulla for the ages used in the experiments.¹⁶ The epinephrin was injected intraperitoneally, usually into litter-mates of the control animals. The mitotic rate was reduced by the injections to a fraction of the normal, one-half to one-twenty-fifth. Excess of epinephrin therefore not only inhibits the function of the individual cells but prevents the formation of new cells.

In the work that we shall now describe we studied the effects of extracts of an organ, the kidney, not known to produce an internal secretion. In the previous experiments hormones were available for the tests. In the case of the kidney hormones were neither available nor known to exist. However, in our preceding work a method had been established for determining the effect of injected substances on the proliferative activity of the tissues of the recipient animal. In the experiments that follow extracts were prepared from the rat kidney and the effect on mitotic rate was determined in the kidney of rats injected intraperitoneally with the extracts. In all experiments a single intraperitoneal injection of the quantity indicated was made and the animal killed twenty-four hours later. At first simple aqueous extracts were prepared and tested. Then less simple extractions and fractionations were carried out. A uniform method

of testing the extracts was used in all experiments.¹¹ Rats unweaned, about 25 days of age, were chosen. Litter-mates were used as controls, and these with the rats used for testing a particular extract or eluate, are given consecutive numbers in the tables to indicate each group. Members of the different litters showed a wide range in mitotic rate. The rats were kept on a standard complete diet and no change in their environment was made at the time of the experiment.

All animals were killed and the right kidney fixed immediately in formalin for paraffin embedding and for sectioning. A transverse block 3 mm. thick was cut from the kidney midway between the poles. Ribbons of sections cut at 8 microns were mounted from the center of this block and stained. The mitoses present in the renal tubules in three to five sections were enumerated.

The extracts were prepared from fresh kidney and from desiccated kidney of full grown rats. The etherized animals were bled by slitting the great vessels of the neck. With aseptic precautions the fibrous capsules were stripped from the kidneys and the capsules, together with the renal vessels and all peripelvic fat and fibrous tissues, were removed. The kidneys were then ground thoroughly in a mortar and the ground mass spread on a glass plate for desiccation, which was accomplished by playing an electric fan on the plate for about two hours. By spreading in a very thin layer the pulp was reduced to a hard scale within five minutes. The material was scraped from the glass with the aid of a safety razor blade and ground to a fine powder in a mortar. It was then placed in a bottle over phosphorous pentoxid and the air exhausted. Various extracts were prepared from the desiccated kidneys.

SIMPLE AQUEOUS EXTRACTS OF FRESH MACERATED KIDNEY AND OF DESICCATED KIDNEY (TABLE I)

Extract 1: To 3.2 gm. of fresh kidney pulp 16 cc. of distilled water were added and the supernatant fluid collected by centrifugation. Large doses yielded inconclusive results (Rats 1-4).

Extract 2: To 1.3 gm. kidney desiccate 13 cc. of distilled water were added. The mixture was centrifuged and the supernatant fluid collected. This extract reduced the mitotic rate to a fraction of that of the controls (Rats 10-12).

Extract 3: To 1.4 gm. kidney desiccate 28 cc. of distilled water were added and the supernatant fluid collected as in the other extracts. In this experiment the number of mitoses was reduced (Rats 20-22).

Extract 4: This extract was made by filtration of the supernatant

TABLE I

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
1	6 cc. Extract 1	days 26	3.0
2	6 cc. Extract 1	26	1.0
3	Control	26	8.2
4	Control	26	3.0
10	4 cc. Extract 2	27	0.2
11	Control	27	9.2
12	Control	27	7.2
20	4 cc. Extract 3	25	5.8
21	5 cc. Extract 3	25	4.0
22	Control	25	17.2
30	0.5 cc. Extract 4	25	4.2
31	1 cc. Extract 4	25	8.4
32	2 cc. Extract 4	25	2.4
33	4 cc. Extract 4	25	2.8
34	Control	25	23.0
35	Control	25	16.6

fluid from kidney desiccate through filter paper several times. The supernatant fluid was prepared as in Extract 2. Although coarse particles in suspension were removed by the filtration the inhibitor was not materially lessened (Rats 30-35).

FILTRATES OF SIMPLE AQUEOUS EXTRACTS OF DESICCATED KIDNEY (TABLE II)

Our next step was an attempt to remove by filtration all cell particles held in suspension. The extracts of the filtrates were made

TABLE II

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
1	4 cc. Extract 1	<i>days</i> 23	11.6
2	Control	23	37.8
3	Control	23	44.0
4	Control	23	28.8
10	2.7 cc. Extract 1	25	22.2
11	2.7 cc. Extract 1	25	22.6
12	Control	25	36.0
20	2.5 cc. Extract 1	24	15.2
21	Control	25	15.4
30	4 cc. Extract 2	28	0.8
31	Control	28	0.7
40	5 cc. Extract 3	25	33.6
41	Control	25	24.4
50	4.25 cc. Extract 3	24	12.5
51	Control	24	12.8

as given in Table I, except that the ratio of water to desiccate was somewhat increased.

Extract 1: To 1 gm. of kidney desiccate 20 cc. of distilled water were added and the supernatant fluid collected as before. This extract was passed through a Mandler bougie. There was a reduction in mitotic rate of only 17 per cent. In a second extract there was practically no reduction.

Extract 2: This was prepared as in Extract 1, except that the fluid was passed through a Berkefeld N filter. The inhibiting principle was removed.

Extract 3: An attempt, which was unsuccessful, was made to adsorb the inhibiting agent contained in Extract 1 (Table I) by aluminum hydroxid. The aluminum hydroxid designated by Wilstätter and Kraut¹⁷ as Type C was employed. Murphy and co-workers¹⁸ used this preparation to adsorb the active principle of Rous Chicken Tumor I. Later Murphy and Sturm¹⁹ determined

TABLE III

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
1	2 cc. Extract 1	days 24	13.0
2	Control	24	59.5
3	Control	24	40.5
10	2 cc. Extract 2	26	3.3
11	0.3 cc. Extract 3	26	4.6
12	0.125 cc. Extract 3	26	3.6
13	Control	26	3.0

that an inhibiting substance was adsorbed along with the active principle. We recognized in all these experiments that we might be dealing with a combination of stimulant and inhibitor.

ACID-AQUEOUS EXTRACTS OF DESICCATED KIDNEY (TABLE III)

Extract 1: To 2.3 gm. of desiccated kidney 46 cc. of N/1 HCl were added. The mixture was placed in a boiling water bath for one hour. The supernatant fluid was obtained and made just alkaline to litmus with N/1 NaOH. The precipitate obtained by centrifugation was taken up with 3 cc. of distilled water and the solution made neutral to litmus. The total volume was 4 cc.

Extract 2: Made by same procedure as that used in the preparation of Extract 1.

Extract 3: Supernatant liquid above the isoelectric precipitate of Extract 2.

The extractions were made with relatively strong acid and at a high temperature. Both the isoelectric precipitate and the supernatant liquid were tested. In consideration of the large quantity of kidney represented in the dosage the first experiments with the precipitate gave little or no evidence of inhibition (Rats 1-3). A second test of another preparation was negative (Rats 10 and 13). The liquid above the precipitate was toxic in large doses. Sublethal doses did not inhibit (Rats 11-13). A tentative conclusion at this time assumed that the inhibitor was destroyed by the acid, high temperature, or the two in combination.

TABLE IV

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
1	4 cc. Extract 1	<i>days</i> 24	10.0
2	3 cc. Extract 1	24	11.0
3	Control	24	18.3
4	Control	24	11.0

ACID-AQUEOUS EXTRACTS OF FRESH MACERATED KIDNEY (TABLE IV)

Extract 1: To 9 gm. of fresh kidney 18 cc. of 3 per cent HCl were added. The mixture was placed at 70° C for twenty minutes and then filtered through muslin. After neutralization to Congo red with N/1 NaOH an equal volume of acetone was added and the mixture placed in the ice-box overnight. The clear supernatant liquid obtained by centrifugation was reduced to a small volume by fanning and then made up to 21 cc. with water.

The method differed from the preceding one recorded in Table III in several particulars. The extraction was made at a somewhat lower temperature, and fresh minced kidney was extracted. In the quantities used the extract was without effect on the mitotic rate of the renal epithelium.

ACID-ALCOHOL EXTRACTS OF DESICCATED KIDNEY (TABLE V)

Extract 1: To 0.7 gm. desiccated kidney there were added 14 cc. of 60 per cent alcohol made 0.2 per cent acid with HCl. The mixture was kept at room temperature for one hour. The supernatant fluid was obtained by centrifugation. After fanning off the alcohol water was added and the liquid neutralized with N/1 NaOH. Water was then added to make the total volume 5 cc.

Extract 2: Preparation by the method used for making Extract 1. In Extract 1 the strength of acid was reduced and a low tempera-

TABLE V

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
		days	
1	1 cc. Extract 1	24	7.6
2	4 cc. Extract 1	24	9.0
3	Control	24	42.0
4	Control	24	62.0
10	3 cc. Extract 2	24	2.6
11	2 cc. Extract 2	24	2.0
12	Control	24	20.0
13	Control	24	8.3

ture employed. Rats 1-4 inclusive withstood a large dose and the inhibition was pronounced. Another preparation was made and the experiment repeated (Rats 10-13). Again the inhibitory action was decisive.

ACID-ALCOHOL EXTRACTS OF FRESH KIDNEY (TABLE VI)

Extract 1: To 15 gm. fresh macerated kidney 45 cc. of 95 per cent alcohol, 0.36 cc. 95 per cent H_2SO_4 and 15 cc. of water were added and the mixture heated at 40° C for two hours, and at 20°-25° C for three hours. The extract obtained by filtration through muslin

was made neutral to litmus by the addition of N/1 NaOH and the supernatant liquid obtained by centrifugation. After removal of the alcohol by fanning, sufficient water was added to make the total volume 30 cc.

Extract 2: After bringing the pH of Extract 1 to pH 4.8 the concentration of alcohol was made up to 75 per cent. After standing

TABLE VI

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
1	0.1 cc. Extract 1	24	4.0
2	0.16 cc. Extract 1	24	13.0
3	0.2 cc. Extract 1	24	7.3
4	0.2 cc. Extract 1	24	2.3
5	Control	24	45.0
6	Control	24	15.6
10	0.9 cc. Extract 2	25	1.0
11	Control	25	20.0
12	Control	25	18.3
20	1 cc. Extract 2	24	5.3
21	1 cc. Extract 2	24	8.6
22	Control	24	45.0
23	Control	24	15.6

forty hours in the ice-box the precipitate was separated by centrifugation. The supernatant fluid was reduced to a small volume by fanning and made up to original volume with water.

In Extract 1 a large flocculent isoelectric precipitate was removed and the supernatant liquid proved to be inhibitory (Rats 1-4). This liquid was further purified by alcoholic precipitation which removed sulphate and protein. Its inhibitory potency was not affected by the purification (Rats 10-12, and Rats 20-23).

DISCUSSION AND SUMMARY

It is well known that certain organs elaborate chemical regulators or hormones which govern growth and metabolism. It has been demonstrated that several of these inhibit karyokinesis of the cells that produce them. This self-regulatory function of the hormones is not known to depend on their metabolic activity. In the case of the parathyroid the hormone made inactive, as far as calcium metabolism is concerned, still inhibits proliferation of the parathyroid cells. The experiments recorded in this paper describe methods for the extraction from rat kidney of an inhibitor of renal tubule mitosis. It is not known that this inhibiting agent exercises any control over growth of the body as a whole, or that it plays any rôle in metabolism.

Simple aqueous extracts made from the fresh macerated kidney had little influence on the proliferation of renal epithelium. Simple aqueous extracts of desiccated kidney, however, were inhibitory when sufficiently large doses were injected. A more satisfactory potency was obtained by means of acid-alcohol extraction of either fresh or desiccated kidney. The successful extracts were made with dilute acid, 60 per cent alcohol, and at temperatures of 40° C or less. Those acid-aqueous extracts that were tested were without effect on the renal epithelium. They were prepared at 70° C or more and the strength of acid was relatively strong. Further attempts are being made to purify and concentrate the extract.

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THE REACTION TO FINE AND MEDIUM SIZED QUARTZ AND ALUMINUM OXIDE PARTICLES. SILICOTIC CIRRHOSIS OF THE LIVER.*

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The experiments to be described were performed as part of a general program for study of the reaction of the body to various inorganic dusts. Previous investigation had demonstrated that it is possible to reproduce the typical nodular lesions of silicosis in guinea pigs and rabbits by exposing them for 8 hours daily over a period of 1 or more years to the inhalation of fine silica dust.¹ These observations failed to indicate the nature of the injury produced by silica and they did not define the quantity and size of the particles necessary to provoke reaction. The present paper deals with preliminary attempts to evaluate the importance of the factor of size. It also throws further light upon the nature of the injury.

It is well established that the reaction to silica differs from that to any other known type of dust and it has been hypothecated that the biological activity of this substance is due to its solution in the slightly alkaline fluids of the body. This hypothesis is based upon the clinical observations of Collis² and upon certain experiments of Gye and Purdy.³ The latter investigators showed that a colloidal suspension of silica is toxic. Intravenous injection of large doses produces almost immediate death; repeated smaller injections injure various cells and later stimulate proliferation of connective tissue. Subsequent writers have erroneously assumed that the silica sol used by Gye and Purdy was a chemical solution, whereas it was actually a colloidal suspension of exceedingly fine particles. More recently Heffernan⁴ has suggested that silica is active when freshly fractured because of its molecular structure, which presents numerous unsatisfied oxygen atoms to interact with tissue elements. This hypothesis would demand that the reaction produced by a given quantity of silica be proportional to the size of the particles, since a larger num-

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ber of unsatisfied oxygen atoms would be exposed with successive increases in subdivision. Regardless of the mechanism involved all available evidence points to a chemical rather than a physical type of injury by silica.

As originally planned, these experiments were designed to demonstrate the relation of particle size to the amount of reaction that would be produced by a measured quantity of silica. If such reaction were to take place at the surface of the particles it should be proportional to the amount of surface exposed to the body fluids. Since a known quantity of material must be introduced into the body for such a study intravenous injection was substituted for inhalation.

Two series of 8 rabbits each were injected with 1.3 gm. of silica particles. For the first series the particles were approximately the size of leukocytes, being 6 to 12 microns in maximum diameter; for the second, they were about one-tenth as large (1 to 3 microns). To control the action of the silica a third series of 8 rabbits was injected with 1.2 gm. of aluminum oxide particles of the same size as those of the smaller silica fraction (1 to 3 microns). This substance was selected because its chemical properties simulate those of silica more closely than those of any other known substance. The alumina employed was an electric furnace product known as "aloxite." The size of all particles was carefully graded by a process of fractional sedimentation which has been described elsewhere.⁵ Two per cent suspensions by weight were made in physiological salt solution and sterilized in the autoclave. It was originally intended to inject each animal with 10 cc. each week of the experiment, but it soon became apparent that this program could not be followed as the fine quartz particles provoked so much reaction in the ears that long periods of rest between injections were necessitated. As a consequence from 1 to 4 months elapsed before the full 1.3 gm., often in doses as small as 3 cc., could be administered. Seven of the 24 animals died of embolism during the course of treatment and 2 more in the group receiving the finer quartz particles died subsequently of silicosis. One animal died during the first injection and showed coccidiosis, but the others were entirely free from any trace of this infection. The remaining members of the three groups were killed from 2 to nearly 3 years after completing the injections.

EXPERIMENTAL RESULTS

Fine Silica Particles (1 to 3 Microns)

This material is irritating and it tends to produce thrombosis in the ear veins, followed by accumulations of the dust in the surrounding tissues.

Ear: In this location the dust provokes a localized necrosis with an acute exudation of polymorphonuclear leukocytes and a small amount of fibrin. The masses of dust have persisted with little decrease in their size throughout the entire period of observation (1067 days). They become surrounded by a zone of mononuclear leukocytes and this in turn is gradually replaced by connective tissue which is at first cellular and later hyaline in character.

Lungs: The particles of this dust fraction are so fine that only a few of the larger ones are retained in the capillaries of the lungs. They are gradually phagocytosed and transported into the perivascular lymph spaces and thence into the pulmonary lymphoid tissues. A small number ultimately reach the tracheobronchial lymph nodes. The amount of silica in any one spot is so small that very little fibrosis results. Of 2 animals killed at the end of the experiment, 1 shows only phagocytes containing dust particles in the intra- and extrapulmonary lymphoid tissues, and the other shows a limited amount of loose, non-cellular, fibrous tissue in the walls of the air spaces adjacent to the lymphoid tissues.

Spleen: The spleen seems to have filtered out and retained most of the silica particles of intermediate size in this fraction. They are found in large, swollen phagocytes with from one to four nuclei. The number of such particles seen in a section of any one cell cut at 6 microns rarely exceeds eight to ten. There are also smaller particles, only visible with polarized light, which are collected in great numbers by exceedingly large giant cells. A section of such a cell may exhibit as many as thirty or more nuclei massed in its center or arranged in ring formation about its periphery. They are indistinguishable from the Langhans giant cell of tuberculosis.

Three months after completing the injections compact masses of such cells are found in dilated portions of the sinuses. In another 6 months these lesions have increased to nearly 1 mm. in diameter and they often extend through the walls of the sinuses into the septa

and follicles. Blood vessels in the area involved are not destroyed but appear normal at this time. After a year or more the nodules of phagocytes have been replaced by considerably larger nodules of cellular connective tissue exhibiting numerous mitotic figures. They have clearly defined borders and are covered with a thin layer of flat cells, probably sinus endothelium. At their centers the tissue becomes progressively fibrous in character and in the last animal killed a small amount of specific silicotic hyalin has begun to appear. This change is attended by no apparent alteration in the blood vessels.

Liver: The sinusoids of the liver have arrested and retained the major part of the finest particles in this fraction and as these were in great excess, the amount of reaction in this organ is much more pronounced than in any other. No animal was killed sufficiently early to observe the immediate cellular response in this location, but the experiences with larger particles, to be discussed below, would indicate that they are probably phagocytosed by Kupffer cells, which become detached and migrate with their ingested material toward the portal areas. In the first animal killed at 84 days there are numerous multinucleated, dust-containing cells, which are probably still within the sinusoids of the peripheral portions of the lobules. The portal areas are already thickened by proliferating cellular connective tissue and the number of small bile ducts is much more evident than in the normal animal. The lymph vessels in this location are distended with small clumps of dust-containing phagocytes.

The hepatic lymph node shows more marked reaction than the liver at this time. Its medullary sinuses are distended with extremely large giant cells packed with fine dust particles, visible only with polarized light. The afferent lymph vessels are so dilated that their diameter exceeds that of a low power microscopic field. They contain only coagulated lymph.

The next animal, killed at 9 months, shows more dust phagocytes in the sinusoids. Usually they occur in small clusters and are surrounded by a few lymphocytes. The portal connective tissues are now greatly increased in thickness at the expense of the parenchyma, but there is no demonstrable fibrosis within the lobules. At the junction of connective tissue and parenchyma cells there are numerous mononuclear phagocytes but no polymorphonuclear leukocytes. The fibrous tissue is cellular but edematous. The lymphatic spaces are widely dilated and in some places they contain clusters of large

dust-filled giant cells. In addition there are compact clusters of mononuclear phagocytes, frequently in mitosis, which are surrounded by concentric strands of compressed cellular fibrous tissue. These foci contain exceedingly large numbers of minute dust particles. In some instances these lesions have already been replaced by compact nodules of loose cellular connective tissue.

The next 2 animals were not killed until the 656th day, by which time the reaction had progressed to extreme limits. The portal connective tissues have increased tremendously and large groups of lobules have been completely destroyed and replaced. Some portions of the parenchyma appear never to have been involved, while others present evidence of regeneration after injury. The connective tissue is generally non-cellular in character and exhibits clusters of nodular whorls of hyaline fibers staining intensely with eosin. Compact masses of minute, doubly refractile dust particles can be found in the centers of such areas. Elsewhere the connective tissue consists of either heavy hyaline bands or loose edematous fibers. Scattered through it are clusters of small bile ducts and fragments of parenchyma varying in size from a few cells to the central half of a lobule. The liver cells are infiltrated with fat. No polymorphonuclear leukocytes are seen in or about them. Traces of lymphatics and sinusoids have disappeared, but in isolated spaces there are multinucleated, deeply eosinophilic cells, which are probably phagocytes as some of them contain dust particles. As evidence of regeneration there are areas of parenchyma in which no suggestion of lobular arrangement or sinusoids can be discovered. The cells in such areas are pale, non-granular and free from visible fat. No dust particles can be found among them. Uninvolved portions of the parenchyma are apparently normal, except for a well marked fatty infiltration rather sharply limited to the central portion of the lobule.

The hepatic lymph nodes in these animals are completely replaced by compact whorls of dense hyaline connective tissue.

The 4 remaining rabbits examined between the 677th and the 1067th day show only minor variations from the picture just described. Apparently the fibrosis continues to progress with increasing amounts of hyalinization, but some of the less dense connective tissue appears to have been replaced again by regenerating liver cells. In its older portions the fibrous tissue is absolutely devoid of nuclei but where it approaches large blood vessels, in the capsule for

example, it becomes markedly cellular with numerous mitotic figures. While all trace of sinusoids has disappeared there are occasional areas, apparently unconnected with large blood vessels, which are filled with living blood cells. The large bile ducts are preserved intact but evidence of bile stasis is furnished by scattered phagocytes filled with yellow-green pigment.

The gross picture is that of a coarse nodular cirrhosis with marked distortion of the organ. Areas 2 to 3 cm. in diameter may be composed wholly of white, leather-like scar tissue. Sometimes spherical nodules, 8 to 10 mm. in diameter, are attached to the surface of the organ by strings of fibrous tissue. In the less involved portions bright red areas of granular liver tissue are furrowed by irregular depressed bands of fibrosis. In the animals killed later the regenerated portions of the organ project as smooth, dark red masses elevated above the older, gray, nodular, cirrhotic areas.

Larger Silica Particles (6 to 12 Microns)

The wider variation in the size of the particles used in this group is responsible for involvement of different organs. The larger particles are caught in the pulmonary capillaries; some of the smaller ones are carried to the spleen and liver. Not enough are retained within the latter organ to provoke significant reaction, but they are removed by the lymphatics to the hepatic lymph node near the head of the pancreas.

Lungs: In the lungs large fragments of quartz are phagocytosed by mononuclear and giant cells, which collect within the thickness of the alveolar septa. By 180 days after the last injection they have formed nodules that vary in size from only a few cells to that of a distended alveolus. A few particles are transported to adjacent lymphoid tissues. At this time silver impregnation shows fine reticulum fibrils extending from the surrounding connective tissue between the mass of cells. In the course of years these nodules may increase in size and perhaps in number, but in the majority of cases the progression of such lesions is insignificant. In 1 of 3 animals killed after 3 years the nodules are somewhat larger and considerable numbers of dust-containing phagocytes are seen within the intrapulmonary and tracheobronchial lymphoid tissues. However, none of the lung nodules has developed any fibrous characteristics. The

phagocytic cells are still round and although the reticulum fibers are more abundant and somewhat thicker no collagen has developed.

Spleen: In the sinuses of the spleen the polarizing microscope shows considerable numbers of smaller quartz particles (6 to 8 microns). They are included within the cytoplasm of extremely large giant cells occurring in compact clusters within the sinuses. These nodules appear to undergo no further change during the maximum period of observation (1130 days).

Liver: In marked contrast to the extreme reaction provoked by the finer sized quartz dust of the first fraction there is practically none in the liver of any animal of this group injected with 6 to 12 micron particles. In gross the organ appeared perfectly normal. In microscopic sections there is an occasional giant cell containing a particle caught in a sinusoid, and very rarely a small cluster of such cells is found in the portal connective tissues. As time has progressed the only additional feature found is the appearance of a small number of lymphocytes about the dust-containing cells.

The hepatic lymph node shows much more extensive reaction. Most of the normal elements are replaced by wide sheets of large phagocytes. Moderately coarse dust particles are visible by polarized light. In one or two areas there are nodular collections of phagocytes surrounded by definite bands of collagen. In such foci there are a greater number of smaller dust particles (6 microns +).

Kidney: In neither group injected with silica has there been evidence of the dust or reaction to it in the kidney. If the foreign body reached this organ it was apparently not retained. In the case of the smaller fraction macroscopic nodules developed in the marrow of the ribs, which progressed to the stage of cellular connective tissue without hyaline formation.

Fine Aluminum Oxide

Eight rabbits were given 1.2 gm. of aluminum oxide particles, 1 to 3 microns in diameter, in from five to seven intravenous injections, during a period of $4\frac{3}{4}$ months. When killed, at intervals during the following 22 months, none of them showed gross evidence of actual disease. The only changes in any way suggestive of lesions were patches of pigmentation where the black dust particles escaped into the areolar tissues of the ears, at the site of injection, and a slightly increased prominence of the lobulation of the liver.

As in the case of quartz the particles are distributed throughout the various organs according to their size, but aluminum oxide has a very different effect upon the phagocytes and upon connective tissue. A limited number of phagocytes is adequate to care for the alumina injected because each cell ingests an excessive quantity of particles. So many of them accumulate within the cytoplasm that the internal structure of the phagocyte is often completely obscured. Under favorable conditions several nuclei can be made out within a cell hypertrophied to several times its normal size. Migration is apparently impeded as the giant cells remain scattered throughout the tissue and do not collect in masses as in the case of quartz. Furthermore, ingested alumina particles do not seem to injure the cells for there is no accumulation of polymorphonuclear leukocytes or proliferation of mononuclear phagocytes. Evidence of stimulation of fibroblasts has been entirely lacking throughout the entire period of observation. In fact the only further change observed in nearly 2 years after completing the injections has been a slight accumulation of lymphocytes about the alumina-containing cells.

In the *lungs* there are capillary emboli of dust particles surrounded by a few phagocytes. In the *spleen* isolated mononuclear or giant cells packed with black particles are scattered widely throughout the sinuses, follicles and interstitial tissues. In the *liver* there are giant cells in the sinusoids at the periphery of the lobules and in the portal connective tissues. Twenty-two months after completing the injections the only change that has resulted is a moderate degree of lymphocytic infiltration about these depots.*

DISCUSSION

This experiment has provided new data on the biological activity of quartz particles of various sizes. It has also demonstrated the inability of aluminum oxide, a commonly used abrasive material, to provoke a corresponding reaction. To the experimental pathologist these observations are of interest for two reasons: (1) they demonstrate that insoluble foreign particles injected into the blood stream are filtered out by various organs according to their size, and (2) they

* Similar experiments have been made with *diamond dust*. The quantity injected was less than in the case of silica and alumina but 1 of 5 animals was allowed to survive for 3 years. The distribution and reaction to these extremely hard particles were in every respect comparable to that produced by alumina.

illustrate the capacity of extrapulmonary tissue to react to silica in a characteristic manner. The silicotic cirrhosis produced in the liver is a curious lesion. To the industrial hygienist the contrast between the rapidly progressive reaction produced by very fine quartz particles and the sluggish reaction to coarser quartz and aluminum oxide is of practical significance.

The capacity of the tissues to filter out and localize particles of different sizes in various organs has been the factor determining the distribution of the disease produced by the injected silica. The largest dust particles were apparently retained mechanically in the capillaries of the lungs. Somewhat smaller ones were held back in the spleen while the finest ones were lodged in the liver. The lymphatics of this organ removed as many as possible to its lymph node, situated near the head of the pancreas adjacent to the common bile duct and portal vein. The large number of fine particles provoked such a severe reaction in the node that its function was disturbed; the flow of lymph in the afferent vessels was impeded and the foreign bodies could no longer be eliminated. As a result they accumulated in and about lymph vessels within the liver. This observation constitutes another instance of the importance of the lymphatic system in the development of silicosis. It is of interest to note that whereas in the pulmonary apparatus an obstructive lesion in the tracheobronchial lymph nodes is followed by accumulation of dust in the intrapulmonary lymphoid tissue, in the hepatic system, where there is normally no lymphoid tissue within the viscus, the dust particles accumulate in and about lymphatic vessels. The absence of dust particles and resultant reaction in the kidney is indicative of the lack of a specific mechanism for segregating particulate matter in this organ. These observations also substantiate the conclusions drawn from the inhalation experiments that the reaction to silica is a purely local one about points where particles have been concentrated and that no dissolved silica circulates in the blood stream to damage remote organs. These findings may be of clinical significance because of the disputed contention that the incidence of nephritis is excessive among the victims of silicosis.

A comparison of the reaction to fine quartz with that to aluminum oxide particles of the same size demonstrates the marked difference in the behavior of the phagocytes. The same cell will continue to ingest alumina particles until it becomes so overloaded that its struc-

ture is completely invisible and its power of locomotion is apparently lost. In the case of silica comparatively few particles are usually ingested by any one cell; wherever the number of very fine particles is excessive the cells hypertrophy and form huge giant cells. Either because there is no overloading and no mechanical interference with ameboid activity, or because the irritating particles stimulate migration, the silica-containing cells tend to collect in aggregates. Cells containing alumina, on the other hand, remain more scattered throughout the tissue. This difference in the distribution of the phagocytes ingesting the two types of dust has been maintained throughout the 2 to 3 year observation period of this experiment, and it has been repeatedly observed in other investigations where comparative reactions to quartz and other materials were being studied. It appears to be one of the fundamental factors in determining the outcome of the various forms of pneumoconiosis.

The importance of the factor of particle size in producing reaction to silica is the chief contribution of this experiment. It has been shown that large particles, 10 to 12 microns in diameter, provoke a foreign body reaction in the lungs which persists without much further change for nearly 3 years. Smaller ones, from 3 to 6 microns in diameter, cause slowly progressive changes in the spleen and hepatic lymph node. Very fine ones of the order of 1 micron in size result in progressive proliferation of connective tissue in the liver.

It might be objected that the number of large particles localizing in any one area was not sufficiently great to provoke reaction, but this objection is met by the findings in the spleens illustrated in Figures 11 and 12. It will be noted that the relatively large number of particles, 6 to 8 microns in diameter, have provoked only a reaction of phagocytes 3 years after their injection, whereas the much smaller number of fine particles, 2 to 3 microns in diameter, have caused an extensive proliferation of connective tissue. It therefore seems justifiable to conclude that fibroblastic proliferation occurs because of the surface area and not because of the mass of silica in a given area.

Space does not permit a discussion of the histogenesis of the silicotic nodule but this subject has already been covered in the previously mentioned paper on silica inhalation.¹ It may be stated here that the original nodule of phagocytes, formed by migrating cells, increases in size as a result of mitotic division. The silica concen-

trated in the center of the mass kills many of the cells and this excites a moderate amount of leukocytic infiltration. At the same time fibroblasts are growing into and replacing the original mass, so that it now comes to resemble an actively growing fibrosarcoma. After a year or more this connective tissue becomes fibrous in character and finally a characteristic hyaline swelling takes place at the center, involving one fiber after another. Since such lesions often contain patent blood vessels it is believed that hyaline formation is due to the toxic action of silica rather than to lack of nutrition.

Cirrhosis of the liver developed in the group of animals injected with very fine quartz dust because sufficient quantities of active silica particles were caught and retained in its stroma. No cirrhosis resulted from the larger quartz particles because most of them were removed by the lymphatic system and the few that remained were too large to be active. No cirrhosis was produced by the injection of the same quantity of fine aluminum oxide particles because they are incapable of exciting a progressive fibrosis.

The part played by the phagocytes and the lymphatic system in the concentration of silica particles has already been discussed. The presence of a sufficient quantity of this irritant in the lymphatics of the portal connective tissues set in motion a progressive proliferation of fibroblasts in their immediate vicinity. Increase of the stroma took place at the expense of the parenchyma of the organ until large portions of the functioning elements had been replaced. Whether the liver cells were destroyed wholly as the result of pressure or whether they were first injured by the toxic action of the silica is not clear. There was marked fatty infiltration, but in only one animal, killed on the 980th day, was there any marked infiltration with polymorphonuclear leukocytes. In the inhalation experiments guinea pigs, but not rabbits, exhibited a well marked metastasis of inhaled dust to the liver and in this location there was a well marked leukocytic infiltration. In these animals injected intravenously only mononuclear leukocytes were found between the advancing edge of the fibrous tissue and the parenchyma. Whether leukocytic infiltration is a feature of the process which was not observed because the animals were not killed at proper intervals is not known.

The overgrowth of fibrous tissue between and within the liver lobules resulted in an extreme grade of coarse nodular cirrhosis of macroscopic proportions. It was apparently much more marked

than the lesion that Gye and Purdy produced with intravenous colloidal silica, but the amount of silica introduced in our experiments was greater and the animals survived considerably longer. The later stages of the picture were further complicated by regeneration of extensive portions of the parenchyma. This furnishes some extremely bizarre gross pathological pictures. Apparently a sufficient portion of the organ continued to function in most instances to maintain the circulation, as only 1 rabbit developed ascites. This was in an animal that died on the 656th day.

SUMMARY AND CONCLUSIONS

Three series of rabbits were injected intravenously with 1.3 gm. respectively of silica particles 1 to 3 microns in diameter, silica particles 6 to 12 microns in diameter, and aluminum oxide particles 1 to 3 microns in diameter. The injections were given in divided doses and required from 1 to 4 months for their completion.

These particles were segregated in different locations according to their size. The largest ones were caught in the pulmonary capillaries, those of intermediate size in the spleen and hepatic lymph node, and the finest ones in the liver.

Fine silica particles are most active and have produced a progressive, coarsely nodular cirrhosis of the liver attended by extensive destruction of the parenchyma, followed later by regeneration in certain areas. This cirrhosis is the result of a typical, hyaline, nodular, silicotic fibrosis originating in the portal connective tissues.

Coarse silica particles, 10 to 12 microns in diameter, are much less irritating. They excite a simple foreign body type of reaction which has progressed very little in practically 3 years time.

Fine aluminum oxide particles of the same size as those of the smaller silica fraction and introduced in essentially the same quantity have been merely phagocytosed and have produced no fibrosis in the stroma of any organ where they were deposited.

These observations support the viewpoint that the injury produced by silica is specific and chemical rather than physical in character.

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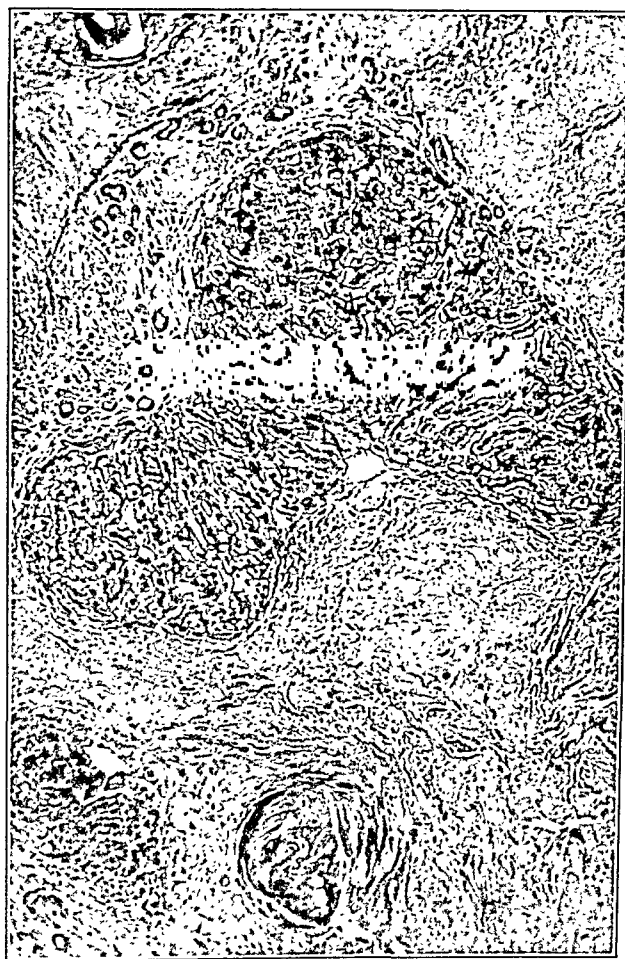
DESCRIPTION OF PLATES

PLATE 120

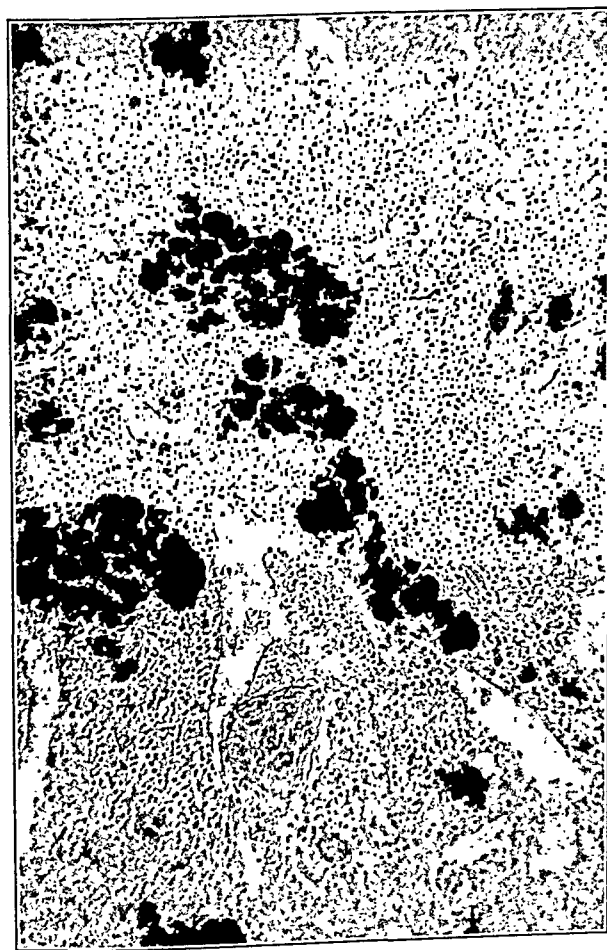
- FIG. 1. Silicotic cirrhosis of the liver. Animal killed 1067 days after completing injections of silica. Upper portion shows nodular external surface of organ. Lower portion shows cut surface. Note large nodule composed wholly of scar tissue in the center. $\times 1.66$.
- FIG. 2. Silicotic cirrhosis produced by 1 to 3 micron silica particles. Animal killed at 655 days. Only a portion of several liver lobules is left in the center of an extensive fibrosis. $\times 100$.
- FIG. 3. Spleen of rabbit injected with 1.2 gm. alumina particles 1 to 3 microns in diameter. Killed in 656 days. Note the lack of reaction about dust-filled phagocytes. $\times 150$.
- FIG. 4. Liver of rabbit injected with 1.2 gm. alumina particles 1 to 3 microns in diameter. Killed at 1095 days. Compare with Fig. 2, showing reaction to the same amount of the same sized silica particles. $\times 150$.



1



2



3



4

Gardner and Cummings

Reaction to Quartz and Aluminum Oxide Particles

PLATE 121

FIG. 5. Nodule of dust phagocytes within a band of beginning fibrosis in the portal area. Animal injected with 1 to 3 micron silica particles. Killed after 275 days. $\times 200$.

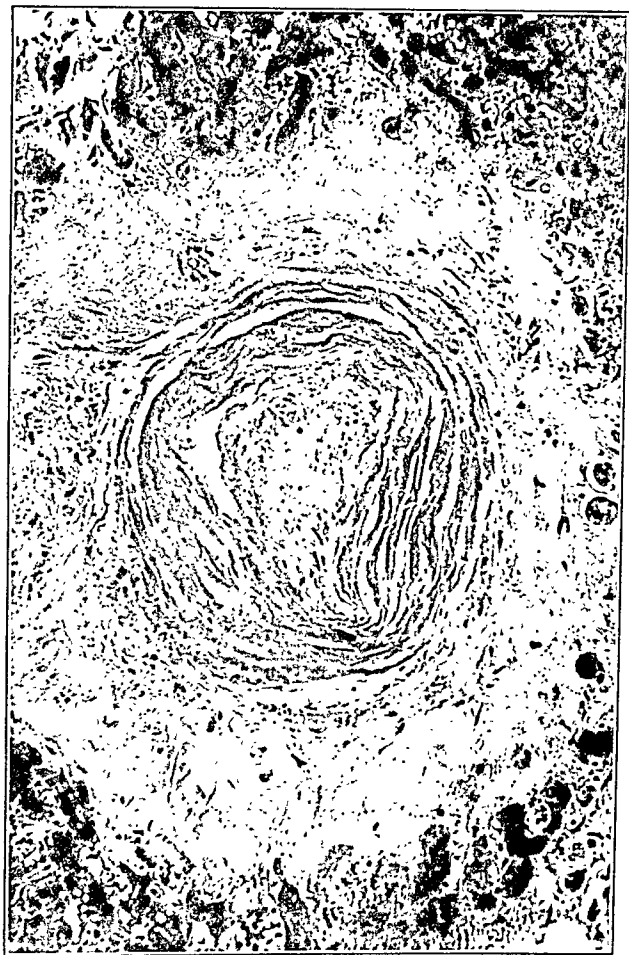
FIG. 6. Hyaline transformation in a nodule similar to that shown in Fig. 5. $\times 200$.

FIG. 7. Extensive nodular hyaline fibrosis in portal area. The centers of the nodules are filled with fine dust. No nuclei are left. Animal killed after 655 days. $\times 100$.

FIG. 8. Regenerated parenchyma in a silicotic cirrhosis. Animal killed 677 days after injections. $\times 100$.



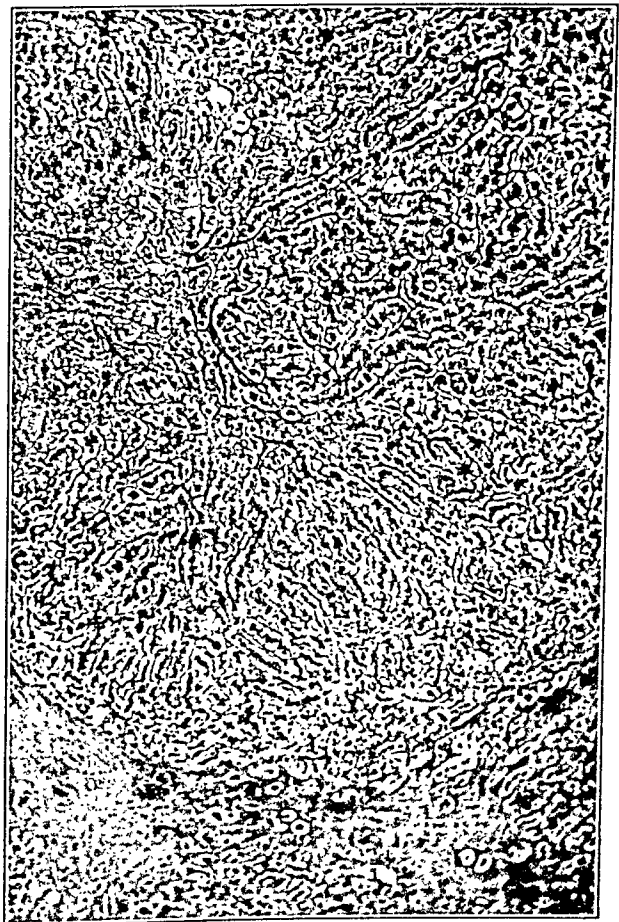
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PLATE 122

FIG. 9. Lung of rabbit killed 180 days after the injection of 1.3 gm. of 6 to 12 micron silica particles. Note the foreign body giant cell reaction with a minimum amount of reticulum. Foot's silver impregnation method. $\times 200$.

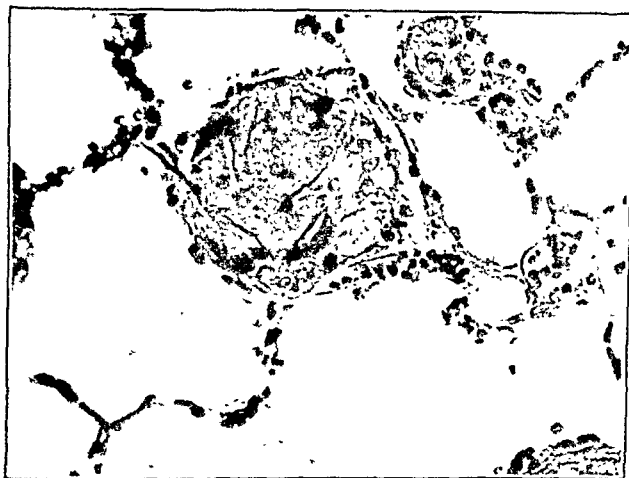
FIG. 10. Another animal of the same series shown in Fig. 9, but not killed until 1130 days after injection. Note the relatively slight increase in size of the nodule and of the amount of reticulum. $\times 200$.

FIG. 11. Spleen of rabbit killed 1067 days after injection of 1.3 gm. of 1 to 3 micron silica particles. Fibrous nodule with very early hyalinization of a few fibers is seen. This reaction is progressive. $\times 95$.

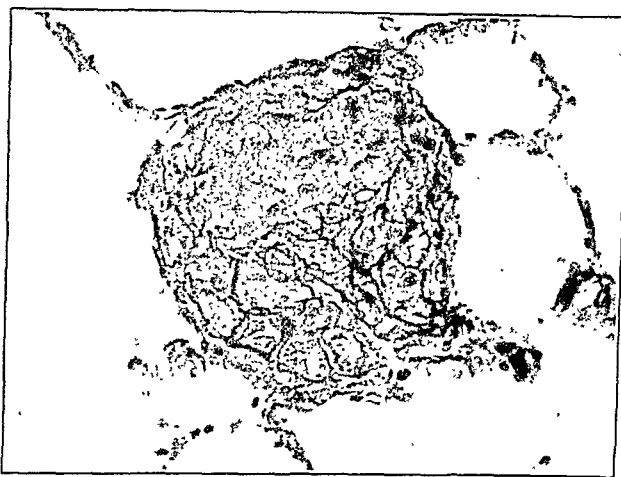
FIG. 12. Spleen of rabbit injected with the same quantity of silica particles 6 to 12 microns in diameter and killed 1130 days after injection. This reaction is non-progressive and consists only of nodules of phagocytes. $\times 95$.

FIG. 13. Silica particles from nodule shown in Fig. 11, seen by polarized light. $\times 240$.

FIG. 14. Silica particles from nodule shown in Fig. 12, seen by polarized light. $\times 240$.



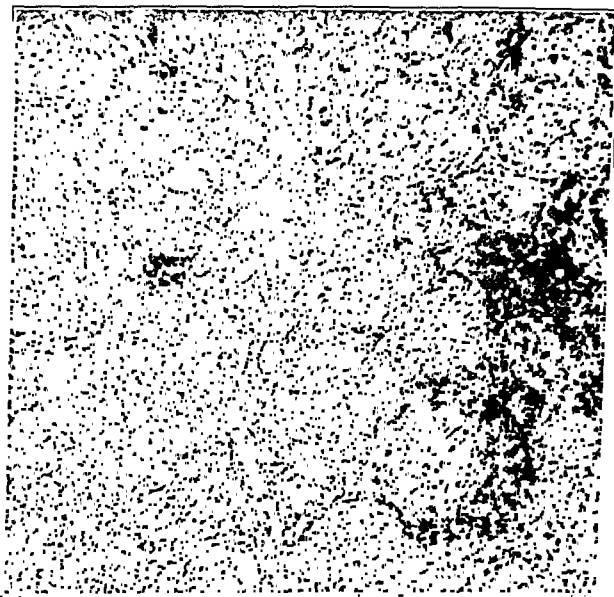
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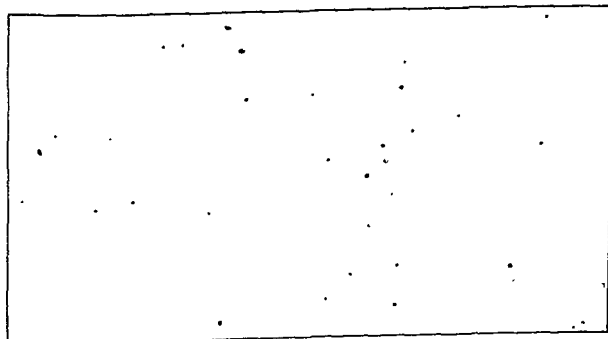
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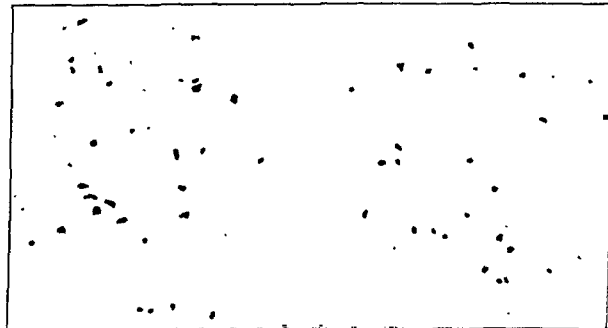
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A GROUP OF METAPLASTIC AND NEOPLASTIC BONE-AND CARTILAGE-CONTAINING TUMORS OF SOFT PARTS *

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Boston, Mass.)*

The occurrence in soft tissues of tumor-like masses containing bone and cartilage has been recognized since the time of Rokitsansky. However, the origin of these structures — more particularly of the extensive and frequently dramatic ossifications of muscle following trauma — has been the subject of an immense volume of speculation, of clinical and pathological observation and of ingenious experimentation, which has recently been well summarized by Koehnlein.¹ Three theories appear worthy of consideration.

1. Cahen² in 1891 suggested a neoplastic origin. Though certainly to be discarded in the great majority of cases of ossification in muscles, tendons and ligaments, it must be granted as established in such cases as the frankly malignant, bone-containing neoplasm reported as Case II by Rhoads and Blumgart.³

2. Origin from periosteum may well explain the type of ossification of tendons, usually described as spur formation, which is always directly connected with bone and usually follows injury or exceptionally heavy functional strain. Examples of this type showing spur formation from the olecranon into the triceps tendon in tennis players and boxers have been described by Baetzner.⁴

Periosteal displacement has been and still is warmly advocated as the cause of so-called myositis ossificans, and the less well known tendonitis ossificans, by many authorities from Berthier⁵ and Lexer⁶ down to the present day. Clinical arguments in its support are the frequency of preceding trauma, the points of predilection in muscles with large bony attachments, and the frequent roentgenographical evidence of periosteal involvement. The experiments of de Josselin de Jong and Eykman van der Kemp,⁷ and many others, in transplanting bone and periosteum into the soft parts offered apparent confirmatory evidence. Nevertheless, all possibility of

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periosteal displacement can be ruled out in many instances. This is particularly true for instance of the numerous cases of ossification of laparotomy wounds, such as the one carefully described by Gruber,⁸ and of the cases of congenital generalized myositis ossificans in children, where clinical palpation of the tissues has been followed by ossification.

3. Origin by metaplasia from fibrous tissue was first suggested by Virchow,⁹ who believed that all bone and cartilage — both normal and abnormal — arose by metaplasia from fibroblastic elements. He regarded osteoblasts and chondroblasts merely as modified fibroblasts. This view, though never universally accepted, has always had its warm proponents — notably Mallory,¹⁰ and still more recently Leriche and Policard.¹¹ So far do the latter authors swing the pendulum in applying the doctrine of metaplasia to bone and cartilage formation that they regard osteosarcomas merely as fibrosarcomas passively ossified by the local conditions of their environment. In favor of this view may be cited the ossification occasionally seen in leiomyomas, neurofibromas and colloid adenomas of the thyroid. Not only does metaplasia offer a simple and satisfactory explanation for the ossification of muscle, tendons and ligaments, but it appears to be the only possible explanation of the many other types of heterotopic bone formation constantly met by the pathologist, such as ossification of arteries, heart valves, of pericardium and pleura, of obsolete tuberculous foci, or of the falx cerebri, to mention only the commoner types.

From clinical and pathological observation metaplastic bone formation can fairly definitely be divided into two types. In one, frank and often long-standing calcium deposits are slowly organized into bone by the fibroblasts of the immediately adjacent tissue. In the other, the formation of cartilage or osteoid tissue either precedes or occurs simultaneously with the deposit of the calcium, as in many cases of myositis ossificans. The mode of ossification in this type parallels with great fidelity normal bone formation in intra- and extra-uterine life, and like it may be either direct from fibrous tissue or secondary to cartilage formation. That combinations of the two types might occur it would be rash to deny.

Experimental production of both types has been successful. The ligation of the renal artery in rabbits is followed by calcification of the degenerating renal tissues and subsequent ossification (Sacer-

dotti and Frattin,¹² Liek,¹³ and others). Ossification of the second type has been achieved by Gruber¹⁴ through traumatization of muscles in rabbits, and by Leriche and Policard¹⁵ in especially constructed tubes of granulation tissue. These experimenters obtained their greatest success if at the same time they produced an experimental destruction of bone in an adjacent area. They feel that two factors are necessary for metaplastic bone formation — young, actively proliferating fibroblasts, which have retrogressed toward the embryonic state, and an excessive supply of calcium, which an adjacent focus of bone necrosis supplies.

From the point of view of differential diagnosis, clinical, X-ray or histological, lesions of the first type rarely offer difficulty. In contrast, lesions of the second type, if caught at the stage of active fibroblastic proliferation, before extensive calcium deposits have occurred, may be extremely difficult to differentiate from neoplasms, either benign or malignant. The following group of cases includes various stages and types of chondrification and ossification in soft tissues, both metaplastic and neoplastic, which offered problems of diagnosis and interpretation sufficiently interesting to seem worthy of record.

CASE REPORTS

CASE I.* *Clinical History:* R. H., female, aged 28 years, unmarried, in good general health, had noted occasionally for the preceding year a peculiar tired feeling in the right hand after writing or exercise. Three or four weeks before entry tenderness developed over the right hypothenar space. Flexion of the fourth and fifth fingers gradually became so painful that she could not use her hand and it was immobilized by a physician. The condition became steadily worse, the tenderness and swelling over the palm increasing, and swelling appeared also on the dorsum of the hand between the fourth and fifth metacarpals. Pain was severe enough to keep her awake at night and to require morphia.

The past history was negative except for a compound fracture of the right elbow four years before, which had drained for several weeks before healing. The hand had not been injured at that time.

The physical examination was negative, except for the local findings. The ulnar third of the palm, particularly over the region of the hypothenar space, showed moderate redness, increased warmth, marked swelling and acute tenderness. Active or passive flexion of ring and little fingers was limited. There was slight swelling and tenderness on the dorsum of the hand between the fourth and fifth metacarpals.

X-ray examination one week after onset was entirely negative. At the time of entry there was evidence of soft tissue swelling. One small linear shadow

* Case of Dr. Arthur W. Allen.

medial to the upper extremity of the fifth metacarpal was noted but was thought to have no pathological significance. The bones and joints showed no evidence of disease.

In the hospital, before operation, a slight fever was noted, reaching 99.5° to 100° F every day. White counts were 12,400, 14,900 and 7400. The differential count showed 63 per cent polymorphonuclears. Since no improvement was noted after six days of complete rest Dr. Allen decided to explore. His pre-operative diagnosis was infection of the hypothenar space. Incision was so made as to expose the ulnar nerve over the hypothenar space. A gristly, firm, friable, tumor-like tissue was found filling the space and extending downward into the region of the interosseous muscles between the fourth and fifth metacarpals. A frozen section was made during the operation, which showed an extremely cellular tissue containing both fibroblasts and histiocytes. Mitoses could be identified. Hyaline material thought at the time to be atypical cartilage was present. Although the findings suggested sarcoma this diagnosis was at such variance with the clinical picture that it was not felt warranted and diagnosis was withheld until after examination of the fixed tissue. Since complete resection was impossible without injury to the ulnar nerve and a mutilating and crippling operation, no attempt was made to remove all the material and approximately one-third of it was left undisturbed.

Upon receipt of a diagnosis of probable myositis ossificans from the histological examination of the fixed tissues all thought of further operative interference was abandoned. The local symptoms rapidly cleared. A small tumor was still palpable, which gradually became firmer. X-ray examination six weeks postoperatively showed a sharply circumscribed, densely calcified mass in the region of the persisting tissue. One and a half years later there had been no further change.

Microscopic examination of the Zenker-fixed tissue showed a complex mixture of tissues including degenerating, multinucleated skeletal muscle cells, well formed but non-calcified lamellae of osteoid material, irregular sheets of osteoid enclosing scattered, large, stellate cells, spindle-celled areas of high cellularity with fairly numerous mitotic figures, and large areas rich in collagen where the collagenous bundles were separated by chains of cells characteristic of tissue histiocytes rather than fibroblasts. These areas were histologically similar to some of the less typical benign giant cell tumors of tendon sheaths. They resembled still more closely the granulomatous tumor masses seen in periarticular tissues. No foam cells or giant cells were present, however. Direct formation of lamellar bone from osteoblasts, which show all stages of transition from fibroblasts, was particularly evident and is illustrated in Figures 1, 2 and 3.

Comment: The absence of trauma, the local signs of inflammation, the slight temperature and elevated white count were all indicative

of an inflammatory lesion. The operative finding of tumor-like tissue was a great surprise and the results of an attempted frozen section diagnosis were inconclusive. Even with paraffin sections of the fixed tissues many areas were so suggestive of sarcoma that numerous pathologists who have reviewed the slides have felt unable to rule out malignancy and some were frankly in favor of it.

It was particularly fortunate that there were good reasons for leaving a portion of the growth undisturbed. The subsequent complete calcification of this remnant, the cessation of growth and even slight shrinkage would appear to rule out beyond reasonable doubt malignancy and even benign neoplasia. The similarity of the histological picture to that of Case I, reported by Rhoads and Blumgart, would raise in the author's mind the question whether that case also was not one of metaplasia rather than neoplasia.

As a case of proved myositis ossificans it is interesting in that the successive X-rays demonstrate clearly the absence of significant deposits of calcium until after a lamellar framework of osteoid material had been laid down. An excess of calcium can hardly have been the stimulating factor that initiated the process.

CASE 2. Clinical History:* E. A., female, aged 28 years, four months before entry noticed a "bunch coming up overnight" on the dorsum of the left foot. She could remember no injury. During the interval the mass grew but slightly and was painful only occasionally.

Examination showed a hard, fixed mass over the third and fourth metatarsals of the left foot. It was not tender and the skin over it was not red. An X-ray plate made one month before admission showed a barely visible, non-calcified tumor mass that appeared to cause slight mechanical spreading of the metatarsals. X-ray examination on admission to the hospital showed definite calcification in the tumor and also two small calcified masses apparently unconnected with the tumor but lying near it between the same pair of metatarsals.

At operation, incision through the skin, subcutaneous tissue and some unidentified dense fibrous tissue, exposed a tumor mass about 2 cm. in diameter lying on and apparently arising from the sheaths of the flexor tendons between the third and fourth metatarsals. It was about 2 cm. in diameter, firm, in part bony, in part cartilaginous, and was readily resected. Convalescence was uneventful.

X-ray examination seven weeks later showed a persistent small area of dense calcification between the metatarsals. A year later this same shadow was present, no larger but now showing a bony pedicle connecting it with the third metatarsal.

Histological examination elsewhere was reported as probable osteogenic sarcoma. Histological examination of two slides referred

* Case of Drs. J. W. Elliot and Harold F. Miller.

to us showed the bulk of the tumor to consist of connective tissue, in part densely collagenous, in part myxomatous, extremely cellular, with occasional mitotic figures. The cells between the collagen bundles were in part typical fibroblasts, but many others were unusually large and blunted, with irregular, oval to horseshoe-shaped nuclei and abundant cytoplasm. These often resembled closely or were indistinguishable from histiocytes. Transitional stages from the myxomatous areas into atypical fibrocartilage and from the more densely collagenous areas into hyaline osteoid material were evident. Several trabeculae of well formed, calcified bone were present. No muscle cells and no giant cells were seen.

Comment: This case also showed histologically active bone formation and areas of fibroblastic proliferation with mitotic figures. In addition there were large masses of rather poorly differentiated fibrocartilage. The first impression was that we were dealing with a slowly growing osteogenic sarcoma. Once again, however, the presence of numerous histiocytic elements did not fit well with a neoplastic origin. Multinucleated cells of the tumor giant cell type could not be found. The location ruled out a muscular origin, but lesions indistinguishable from myositis ossificans do occur in tendons and are often classified as tendonitis ossificans. Since, however, the mass was distinctly outside the tendon it seemed more logical to regard it as metaplastic cartilaginous and osseous transformation of one of the granulomatous tumor-like nodules of tendon sheaths. Once again the successive X-rays showing no calcification, slight calcification, and later dense calcification of the persisting remnants prove that the deposit of the framework of cartilage and osteoid preceded the deposit of calcium.

CASE 3. *Clinical History:* K. M., female, aged 61 years, a paranoiac, with extensive hypertrophic arthritis, varicose veins and a ventral hernia. For two years a tumor had been present over the anterior aspect of the proximal phalangeal joint of the fourth finger. X-ray examination showed it to be calcified and to lie in the soft tissues without demonstrable connection with bone. During the two year period of examination it grew very slightly and its X-ray appearance did not change.

It was resected without difficulty under local anesthesia and was found to overlie and involve the tendon sheath of the flexor hallucis longus.

Gross examination showed a pinkish white, partly cartilaginous, partly friable and granular mass measuring 2.7 by 2.5 by 2 cm.

Microscopic examination showed the mass to consist wholly of

cartilage of varying degrees of differentiation with extensive deposits of calcium. No trace of ossification was discovered (Fig. 4).

Comment: The location of this tumor was characteristic of a tendon sheath granuloma. The frequency of myxomatous degeneration in such lesions is well known and the possibility of metaplastic organization into cartilage is recognized. It seems more logical to regard this as a chondromatous metaplasia than as a primary chondroma. It is interesting that in spite of the extensive calcification of the cartilage neither of the two reactions to calcified cartilage usual under normal conditions — ossification or foreign body giant cell reaction — had occurred.

CASE 4. *Clinical History:* M. B., female, aged 17 years, a previously healthy girl entered the hospital complaining of a "wart" of three years duration on the volar surface of the right hand, overlying the metacarpophalangeal joints of the second and third digits. She had been treated for verruca vulgaris, presumably in acceptance of her own diagnosis, with intramuscular salt solution and bismuth injections (not in the neighborhood of the lesion) without effect.

Examination showed a subcutaneous swelling covered by thickened, callus-like skin. A preoperative diagnosis of cutaneous fibroma was made. Resection was done under local anesthesia and a nodule 1 by 1 by 0.7 cm. with the overlying skin was removed. On section it appeared to consist of bone with a few areas suggestive of cartilage.

Microscopic examination showed on one surface of the tumor pacinian corpuscles and coil glands, and on the other tendon. The bulk of the tumor consisted of trabeculae of well formed bone separated by a vascular stroma containing numerous osteoclasts. At the periphery of the lesion were a few foci of hyaline cartilage. There was no evidence of rapid growth.

Comment: This tumor was so superficial that it lay in part actually within the skin. At its base it backed upon the tendon sheath. The possibility of an extruded sesamoid must be considered but in the absence of any history of present or past functional difficulty in moving the fingers it seems improbable. The histological appearance would suggest that the ossification here was preceded by chondrification and that this process represents the end stage of the same condition described in Case 3.

CASE 5.* *Clinical History:* I. I., female, aged 65 years. For a period of two years the patient was aware of a slowly growing, non-painful tumor of the left thigh. In August, 1929, she consulted a surgeon, who excised the tumor. A

* Case of Drs. S. B. Overlock and R. B. Greenough.

pathological diagnosis of "giant cell tumor" was made upon histological examination. A few weeks later the wound broke down and the tumor recurred locally.

She was seen by Dr. Greenough in January, 1930. At this time he found a large tumor mass, bluish in color, involving the anterior and lateral aspect of the left thigh and extending nearly to the groin, where there were several hard nodes. The abdominal examination was negative.

The possibility of a successful amputation seemed very slight and operation was not advised. By March, 1931, the tumor had broken through the skin, was ulcerated, and had given rise to severe hemorrhage. The patient was losing weight and failing rapidly. The date of her death has not been ascertained but must have been soon after this.

A microscopic slide was sent to us prepared from the original tumor mass. It showed an unencapsulated tumor that was invading the fat tissue at its periphery. Considerable portions of it were necrotic. In the central portions several old calcified areas, some of them strongly suggestive of necrotic bone trabeculae, were observed. The major portion of the viable part of the tumor consisted of masses of giant cells embedded in a cellular, highly vascular tissue composed of spindle cells, apparently typical fibroblasts. Numerous mitotic figures were present in these cells. The giant cells were variable in size, ranging from typical foreign body giant cells with twenty to thirty nuclei to small cells with scanty cytoplasm and three to four nuclei, indistinguishable from tumor giant cells. In occasional areas, particularly in the immediate neighborhood of the old calcium deposits, but also at some distance from them, irregular trabeculae of hyaline osteoid material were found.

Comment: Although the general appearance of this tumor with its immense numbers of foreign body giant cells closely resembled a benign giant cell tumor the frankly sarcomatous nature of the spindle cell masses in which the giant cells were embedded, the presence of cells indistinguishable from tumor giant cells, the rapidity of growth and the invasiveness appeared to leave no question of the present malignant neoplastic nature of the mass. Two possibilities of origin seem reasonable. A fibrosarcoma may have arisen in or near a mass of calcium, possibly an old focus of myositis ossificans or a calcified hematoma. The bone formation might be considered a metaplasia of the fibroblastic tumor elements influenced by the local presence of calcium. This would fit well with the theories of Leriche and Policard. The foreign body giant cells might easily be accounted for by the unorganized calcium masses. The other alternative is to re-

gard the tumor as an osteogenic sarcoma of soft tissues, either primary or possibly secondary to a much older metaplastic process.

The clinical course of the lesion, with the recurrence and the metastasis to the inguinal nodes, leaves no doubt of its malignancy.

CASE 6.* *Clinical History:* S. K., female, aged 55 years. Three months before visiting her physician the patient noticed for the first time a mass in the left breast. It had been painless but it grew rapidly, and by the time she consulted a physician the mass filled the entire breast. There had been no trauma. The skin over the lower aspect of the breast was reddened and at one point fluctuant over an abscess cavity that extended into the tumor.

A preoperative diagnosis of sarcoma of the breast was made and a simple mastectomy performed. The diagnosis was confirmed by frozen section. A radical mastectomy was not thought to be indicated. Convalescence was uneventful. One year later the patient was in good health with no evidence of recurrence.

Gross examination of the excised specimen showed a bulky, spherical tumor 10 cm. in diameter, well circumscribed but not encapsulated, with extensive, central, yellow to white necrosis. There was considerable calcification and a narrow peripheral zone that was pinkish, dotted with yellow and bright red areas, slightly translucent and soft.

Microscopic examination of the viable portions of the tumor showed a highly vascular and cellular mass of spindle-shaped cells, embedded in which were numerous large giant cells of the foreign body type containing ten to fifty nuclei. No cells characteristic of tumor giant cells were found and though numerous mitotic figures were present none of them were multiple. The spindle cells showed well marked fibroglia fibrils with the phosphotungstic acid hematoxylin stain. In many areas in the tumor well formed lamellae of hyaline osteoid material were present and all transitions from typical spindle-shaped fibroblasts to characteristic cuboidal osteoblasts plastered against the freshly forming osteoid could be observed.

Scattered throughout the tumor, but most numerous in the central necrotic zone, were small and large calcium deposits. Some of these were in direct relation to the osteoid material, others were not. Also scattered irregularly throughout the tumor were foci of hemorrhage, deposits of orange-brown pigment readily identified as hemosiderin and also masses of a much brighter yellow crystalline pigment. This latter pigment was suggestive of bilirubin in appearance and color

* Case of Dr. M. P. Smithwick.

and was readily oxidizable to a dark green shade with ferric chloride and trichloroacetic acid (Fouchet's reagent for the identification of bilirubin).

Comment: This tumor, identical in histological appearance with that of Case 5, except for less obvious invasive qualities and somewhat better developed osteogenesis, is, so far as we have been able to ascertain, a unique observation in breast tumors. Though less frankly malignant than that in the preceding case the sarcomatous character of the basic fibroblastic masses, the rapidity of growth and the extensive necrosis, all indicate a neoplasm of indubitable malignancy.

Embedded in this tumor were numerous unorganized calcified masses and scattered granules. It seemed scarcely credible that they were the product of the tumor. A more reasonable view would be that at some period in the past calcification had occurred in the breast as the result of forgotten trauma, or of mastitis with fat necrosis, or of calcification of an adenofibroma. In the neighborhood of this old calcified lesion a fibrosarcoma developed. With increased vascularity partial absorption of the calcium occurred, stimulating a giant cell reaction and also the deposit of osteoid by the embryonic fibroblasts of the tumor.

DISCUSSION

Cases 1 and 2 are particularly interesting because of the similarity of the histological picture, although Case 1 obviously was derived from the interosseous muscles of the hand, and Case 2 from a tendon sheath without involvement of muscle. Both showed sufficient evidence of rapid growth and sufficient atypicality of osteogenesis to be histologically strongly suggestive of neoplasm. In each case a succession of X-rays showed clearly that the active phase of metaplasia occurred before the deposition of significant quantities of calcium, and the source of the stimulus to metaplasia must hence be sought elsewhere.

Cases 2, 3 and 4, because of their attachments to the exterior of tendon sheaths in regions where the so-called xanthomas of tendon sheaths characteristically occur, appear most logically to be explained as phenomena of metaplasia in these nodules. In all of these there was evidence of a cartilaginous transformation preceding ossification. In fact, in Case 3 no ossification whatever was present,

although the cartilage was extensively calcified. In Case 4 at only a few areas on the edge of the tumor did a persisting rim of cartilage give evidence of its earlier stage.

Cases 5 and 6, essentially similar in histological structure, though occurring respectively in the thigh muscles and in the breast, both showed in the central portions of the tumor masses of unorganized calcium presumably antedating the appearance of the neoplasm or at least its active phase of growth. Although in each instance the presence of innumerable huge foreign body giant cells lent to the tumor an appearance almost convincingly similar to that of a benign giant cell tumor of bone, the character of the fibroblastic mass in which the giant cells were embedded was in each instance frankly neoplastic and malignant. The presence of lamellar deposits of osteoid material bordered in many instances by typical osteoblasts raised the question of considering the tumors as true osteogenic sarcomas of soft part origin. On the other hand, the findings fitted perfectly with the theory of Leriche and Policard that bone formation in tumors is essentially metaplastic, the requisites, just as in non-neoplastic metaplasia, being available calcium and embryonic fibroblasts. The old calcium deposits would supply the first, the tumor cells the second. We feel that these two cases present evidence suggestive of the correctness of their theory, as applied to at least a certain group of the extraskeletal bone-forming neoplasms.

SUMMARY

The neoplastic, periosteal and metaplastic theories of extraskeletal bone formation have been discussed and illustrative cases presented. The difficulties of distinguishing between metaplastic and neoplastic lesions have been pointed out and reasons given for regarding Cases 1 to 4 as purely metaplastic and Cases 5 and 6 as primarily neoplastic. The possibility of metaplastic processes within neoplasms, suggested by Leriche and Policard as applicable to all tumors of bone, has been shown to be consistent with findings in these two cases.

CONCLUSIONS

1. Many apparent bone- and cartilage-containing tumors of soft parts are in reality of metaplastic rather than neoplastic origin.
2. Extreme care may be requisite to differentiate the active stage

of osteoblastic or chondroblastic metaplasia from malignant neoplasia.

3. Certain bone-forming spindle cell sarcomas of soft parts may more logically be regarded as fibrosarcomas with metaplastic foci of osteogenesis than as true osteoblastomas.

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DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Case 1. One of the best differentiated areas showing lamellae of non-calcified osteoid material bordered by osteoblasts. $\times 50$.

FIG. 2. Case 1. The edge of an actively forming osteoid lamella, typical osteoblasts and a mitotic figure. $\times 80$.

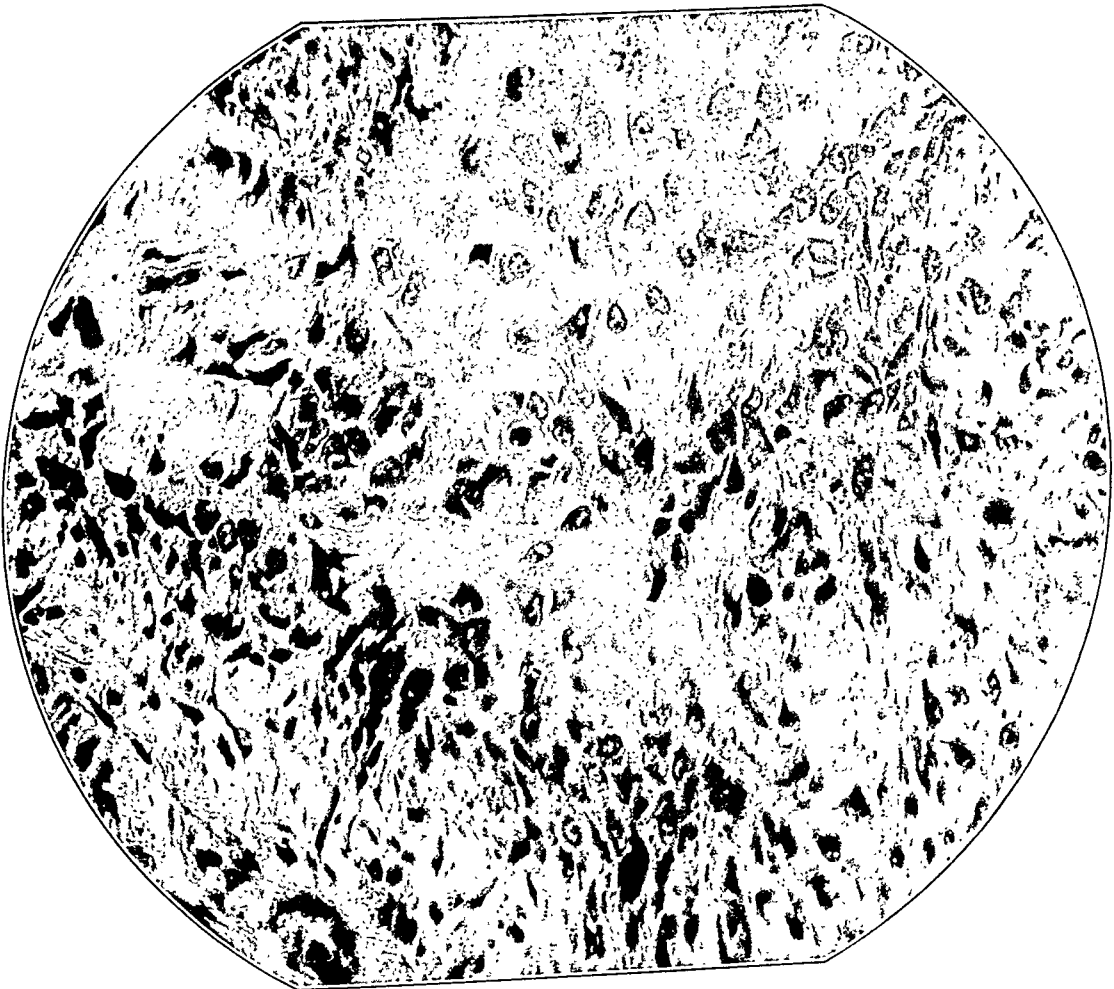
FIG. 3. Case 1. An area of active bone formation showing all transition stages from fibroblasts to osteoblasts. $\times 230$.



1



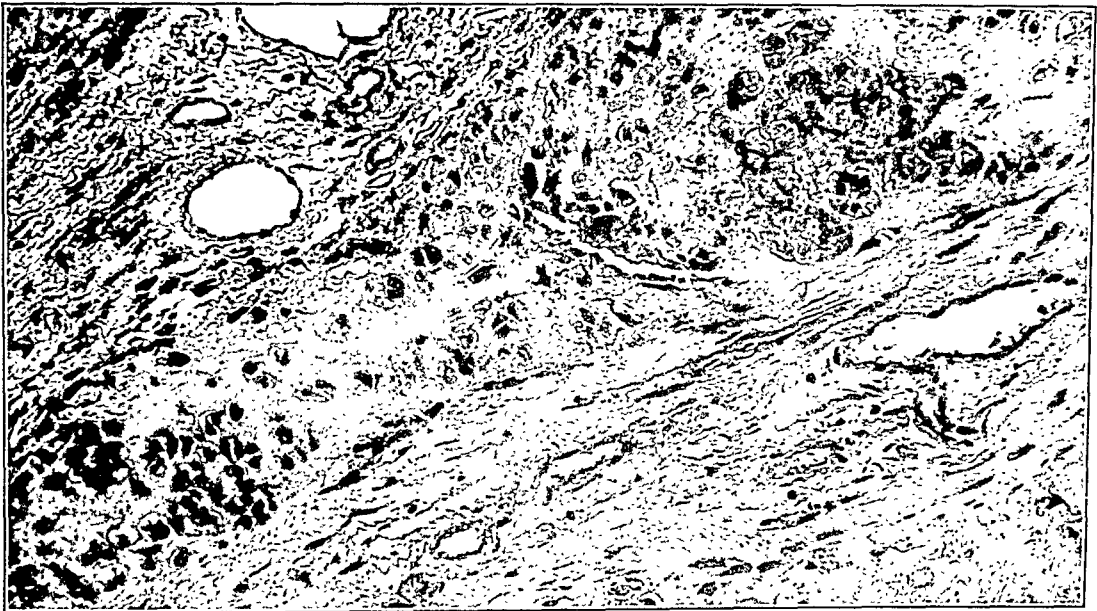
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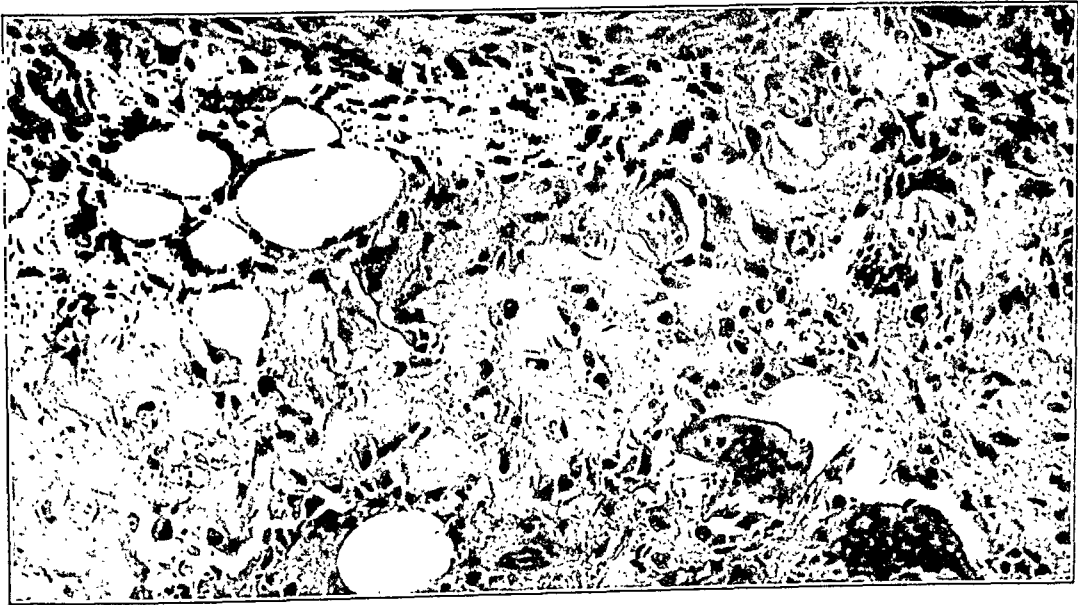
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PLATE 124

- FIG. 4. Case 3. Active formation of hyaline cartilage at the border of the lesion. Zenker fixation has removed the calcium deposits which, in the formalin-fixed material, almost completely hid the underlying cartilage. $\times 150$.
- FIG. 5. Case 5. Sarcoma of the thigh. Giant cells of the foreign body type, hyaline osteoid lamellae, and fibroblastic proliferation. A few large fat cells persist as remnants of the invaded tissues. $\times 200$.
- FIG. 6. Case 6. Sarcoma of the breast. Foreign body giant cells, trabeculae of osteoid and rapidly proliferating fibroblasts. $\times 200$.



4



5



6

A CASE OF CONGENITAL LYMPHOBLASTOMA *

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Instances of congenital neoplasms are extremely rare, and this is particularly true of tumors that arise from the hematopoietic system.

In a survey of the literature we found but four such cases mentioned. De Lange¹ reported a case of congenital lymphosarcoma of the mediastinum in an infant who died at 8 weeks of age. At autopsy a mass was found in the posterior mediastinum, measuring 6.5 by 5.5 by 2.5 cm., which had metastasized to the liver. The blood picture was negative. Eichler² reported a case of congenital lymphosarcoma of the pancreas in an infant 7 days old. The tumor replaced the entire pancreas and measured 8.7 by 5.5 by 5.5 cm. The other organs were negative and no lymph nodes were involved. The family history was unimportant. Another case of primary lymphosarcoma of the pancreas, which appeared in the literature (L'Huillier³), occurred in a 9 day old female infant who died of peritonitis and abscesses of the liver, following phlebitis of the umbilical vein. However, Marxer⁴ and other German authorities seem to agree that what L'Huillier thought to be a primary lymphosarcoma was nothing more than a simple hyperplasia of the normal peripancreatic lymphoid tissue which is quite prominent in infants. Chiari,⁵ in a personal communication, cited a case of generalized Hodgkin's disease in an infant 2 months old, whose mother had had a tumor mass in the axilla that proved, on biopsy before the child's birth, to be Hodgkin's disease.

REPORT OF CASE †

Clinical History: M. A. C., white, female infant, aged 2 months. The family history was negative. The parents and two siblings were alive and well.

At the eighth week of life the infant began to vomit and a profuse

* Received for publication May 1, 1933.

† I am indebted to Dr. James Vance for permission to report this case.

diarrhea was present. Her condition became rapidly more acute in nature, and the abdomen became distended and tender on palpation. It was decided to operate immediately and an exploratory laparotomy failed to reveal volvulus, intussusception, adhesions, or other evidences of inflammation or malformation. The appendix, as will be noted below, presented a most striking appearance and was removed. The child died early the following day.

Permission was obtained for a limited autopsy and the kidneys, spleen, and portions of the intestines were removed.

POSTMORTEM EXAMINATION

Spleen: The spleen weighed 85 gm. and measured 8 by 5 by 2.5 cm. It was enlarged, pale grayish brown, soft, smooth and normal in shape. The cut surface was dark reddish brown, moist, and yielded little pulp on scraping. The trabeculae were poorly defined; the germinal centers were prominent as glistening gray specks averaging 2 mm. in diameter.

Kidneys: The kidneys were similar in appearance. Each weighed 112 gm. and measured 9 by 5 by 4 cm. They were enlarged, still retained much of their fetal lobulation and were firm, smooth, normal in shape, pale gray, and flecked with punctate hemorrhagic areas about 2 mm. in diameter irregularly distributed over the capsular surface. The cut surface showed the normal relation between the pyramids and cortex poorly defined against a pale, grayish brown background. The cortex was 5 mm. in width and the pyramids more widely separated than perhaps is consistent with normal. The capsule stripped with ease, leaving a smooth, coarsely lobulated, glistening surface marked with the punctate hemorrhagic spots mentioned above.

Intestine: Two small pieces were removed. The larger, apparently jejunum, measured 5 by 2 cm. and the wall was 2 mm. in thickness when opened. The smaller piece was similar in appearance and measured 4 by 2 cm. and the wall was 2 mm. thick. The serosa was a pale pinkish gray and was smooth and glistening. The wall apparently was not thickened. The mucosa was not remarkable, except for what appeared to be numerous, minute, submucous, hyperplastic, grayish lymph follicles, having an average diameter of 1 mm.

Appendix: The appendix was 9 cm. long and 1 cm. in diameter. The serosa was smooth, glistening and a chalky pale gray. The or-

gan was firm and less pliable than usual. On section the wall was thickened and edematous in appearance. The lumen, though present, was at no point more than 1 mm. in diameter.

MICROSCOPIC EXAMINATION

The microscopic appearance of these organs is essentially the same. The normal histological architecture has been materially distorted, if not completely obliterated, by a massive infiltration with cells of the lymphocytic series. The extent of the lesion can best be judged by reference to the photomicrographs. The individual cells of the neoplasm tend to be young lymphocytes or lymphoblasts. The latter present large numbers of mitotic figures. The oxydase reaction is negative. The germinal centers of the spleen and intestine are hyperplastic, while those of the appendix seem to be completely lost in the massive lymphocytic infiltration. The larger vessels show such a preponderance of cells of the lymphocytic series that one is forced to believe that the white count would have been in the hundreds of thousands. It seems highly probable from the evidence at hand that other tissues from this case would have shown essentially the same microscopic appearance as those described.

DISCUSSION

This type of tumor is extremely interesting both from an anatomical and from a pathological standpoint. Clinically the outcome is inevitably fatal. Many interesting academic questions come to mind. Is a picture such as this a true lymphoblastoma or would leukoblastoma be a more appropriate designation? Examination of the other lymphoid organs and the bone marrow would have been of great interest and perhaps would have cleared up many points. Does the disease represent a newgrowth, in the sense that it is truly neoplastic, or is it due to an embryonal imbalance, with a simple overgrowth of one particular tissue — a lymphoid monster, as it were? Do others in the family show any similar lesions, as the mother in Chiari's case did? Were this true, it might suggest a case of true heredity. These four cases are scarcely more than pathological curiosities.

SUMMARY

1. The literature concerning cases of congenital lymphoblastoma has been reviewed.

2. A case of congenital lymphoblastoma has been presented in which the normal histological architecture of the organs has been virtually destroyed by a massive lymphocytic infiltration.

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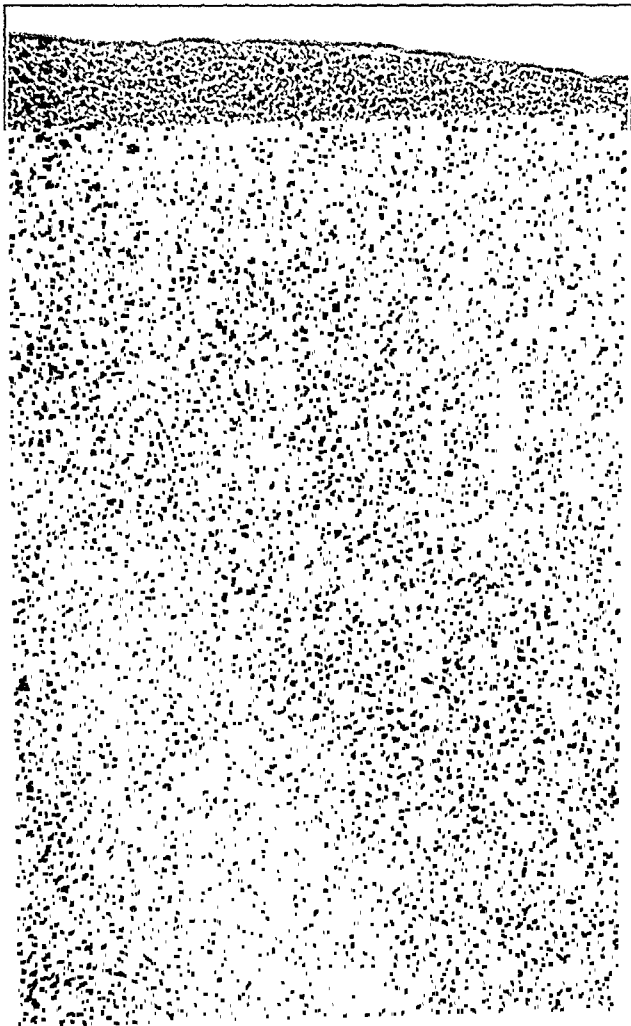
DESCRIPTION OF PLATE

PLATE 125

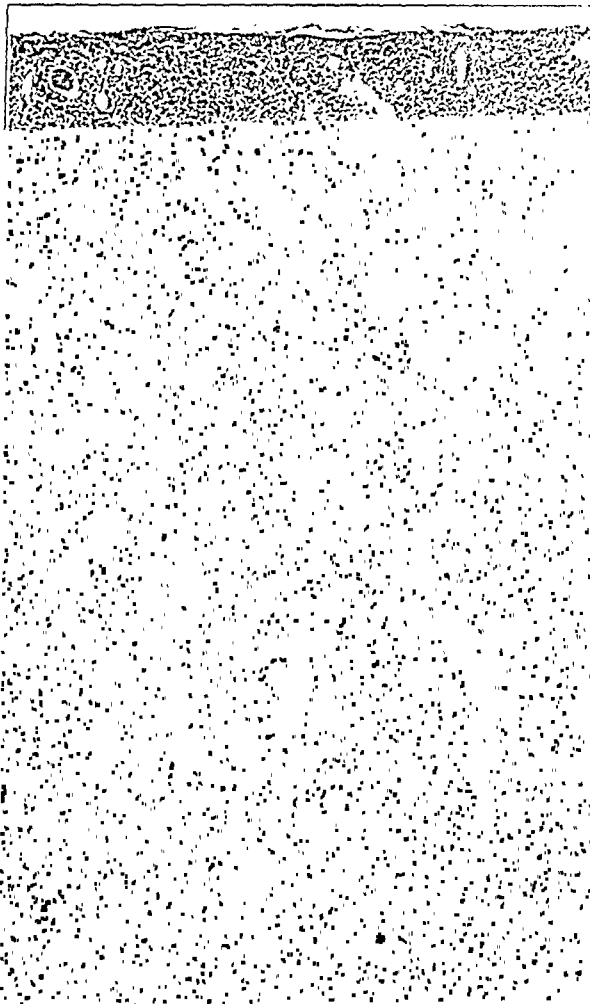
FIG. 1. Spleen. Note massive infiltration with neoplastic cells, almost total obliteration of the sinusoids, hyperplasia of the germinal centers and extreme thinning of the capsule. $\times 15$.

FIG. 2. Kidney. Marked distortion of the histological architecture and infiltration of the interstitial tissue with neoplastic cells is shown. Many tubules are obliterated. Lymphocytes are found rarely within the tubular lumina. $\times 75$.

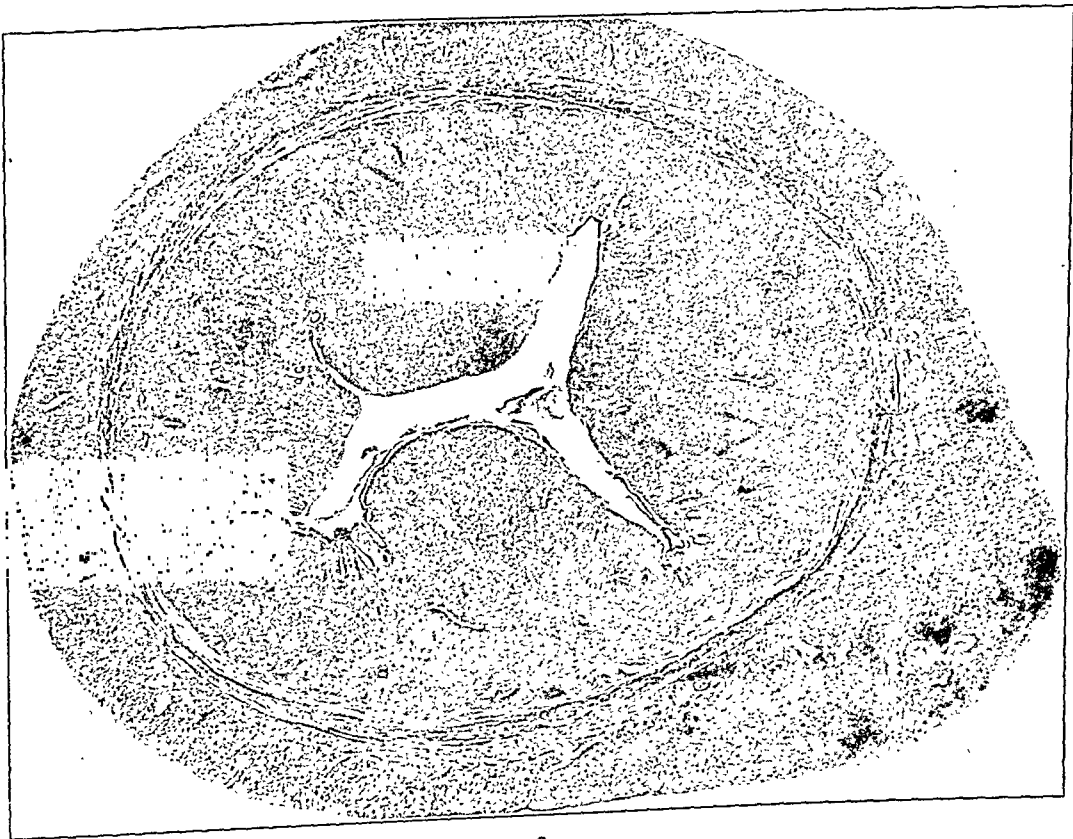
FIG. 3. Appendix. Note complete absence of germinal centers and separation of the muscle fibers with uniform infiltration throughout the entire wall. The mucosa is atrophic but intact. $\times 200$.



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Branch

Congenital Lymphoblastoma

THE ANGLE OF THE MITOTIC SPINDLES IN MALIGNANT CELLS *

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The mitotic spindle has received far less attention than other portions of the karyokinetic mechanism, probably due in part to the emphasis placed on the chromosomes and in part to the technical difficulty of satisfactory demonstration of the spindles. A generation ago Hansemann ¹ called attention to variations in the mitotic figures, including the achromatic spindles of various types of cells.

TABLE I
(Ellermann)

1. GRUPPE

Megaloblasten	18°	$\sigma = 7,2$
Erythrogonien	21°	$\sigma = 6,3$

2. GRUPPE

Lymphoblasten	{ von Leukämie.....	42°	$\sigma = 9,3$
	{ von Leukämie.....	38°	$\sigma = 10,3$
	{ von normalen Organen	39°	$\sigma = 14,0$

3. GRUPPE

Myelozyten	{ neutrophile	66°	$\sigma = 9,0$
	{ eosinophile	73°	$\sigma = 11,0$
Myeloblasten	{ chronische Leukämie	68°	$\sigma = 14,3$
	{ akute Leukämie	69°	$\sigma = 9,7$

Apparently Ellermann ² was the first to give detailed attention to the variation in mitotic spindles and to use it as a means of differentiating cell types. His investigations centered on the megaloblast and the erythroblast, the lymphoblast, the myelocyte and the myeloblast. At first drawing the mitotic figure and then measuring the angle of the spindle from the drawing, he later proceeded to direct measurement, using a goniometer ocular. He found an error of about 5 per cent in measurement with the goniometer. He discovered striking differences between the mitotic spindles of these various types of cells, which are reproduced in Table I, and his conclusions were that "measurement of mitosis is a method that demands a precise technique and skilled experience but has the great advantage

* Received for publication April 5, 1933.

that through a single observation of the cell it gives a mathematical expression available for cell differentiation instead of an inconstant and difficultly demonstrable variation of nuclear structure." Ellermann based his findings on the measurement of at least 40 mitotic angles for each different type of cell. Several years later Petri³ used the same methods on a case of myelogenous leukemia and checked closely with the observation of Ellermann.

I have endeavored to apply these same methods to the study of the mitotic angle in various types of malignant cells. Sources of error are numerous and the difficulty of technique is great. None the less there are certain very definite advantages offered by the method. While perhaps not so valuable as Ellermann believes, as an added means of differentiating cells, and particularly cells whose nature is uncertain, it has certain very definite points in its favor. Thus in cells, the nature of which is controversial, such as those of certain tumors of the thyroid, held by some to be carcinomas, by others to be lymphoblastomas, any means of aiding the differentiation of the types of cell involved is worthy of employment.

Our technique has been as follows. Tissues have been freshly fixed in Bouin's, Regaud's, and Zenker's fluids. In general, our results have been fully as good with Zenker fixation as with any other. For stains we have used Mallory's phosphotungstic acid hematoxylin, Masson's modification of Benda's iron hematoxylin, and Mallory's alum hematoxylin and eosin. The distinct staining of the achromatic spindle seems to be more a matter of vagary of fixation than of subsequent handling of the sections. In general, we have been quite successful in demonstration of the spindles.

The angle measured is that at the centrosome, between the two outer linin fibrils of the spindle. Sometimes the outer fibrils are bowed, and here the general course of the spindle is followed, with due regard to the relation of the centrosome and the chromosomes. If the bowing of the spindle is marked, measurement has not been attempted.

Repetition of measurements of the same angle with the goniometer ocular, working at a magnification of approximately 700 diameters, has shown the percentage of error to lie just below 5 per cent, practically the same as that found by Ellermann. A check by the measurement of enlarged photomicrographs of spindles previously measured with the goniometer ocular has shown less than

5 per cent divergence from the mean measurement obtained by the direct method. Thus, it is fair to say that the use of the goniometer is justifiable and it is a great saving in time and expense.

It is important before measuring a spindle to be sure that the two apices of the spindle lie in the same plane, as any rotation will lead to a false widening of the angle, and also that the equatorial plate is in the same axis as the optical axis of the microscope. Moreover, early stages in the separation of the chromosomes must be guarded against, as these will also produce a widening of the angle. The criteria for suitable spindles for measurement may be laid down as follows: the two centrosomes (more in the case of multiple mitoses) must be visible in the same focal plane; the equatorial plate must be seen edgewise, and there should be no lateral shifting as the focus of the microscope is raised or lowered; the separation of the chromosomes must not have begun.

Measurements were carried out on fifteen malignant tumors, of which six were carcinomas, four fibrosarcomas, two osteogenic sarcomas, one melanoma, one glioblastoma, and one Ewing's tumor.* The results obtained in these tumors are shown in Table II. A few scattered measurements, too few to detail, are included in the summarizing table (Table III).

It will be noted that under the fibrosarcomas two sets of readings are given. In this group of tumors we find two distinct types of mitotic figure. In the regions where the cells are elongate, practically all the mitotic spindles are obviously narrow, with angles less than 90° (Fig. 5). The first set of measurements given in the tables represents the narrow angles; the second set, the wide angles. There is a striking spread, more than 30° , between these two types of angle occurring in the same tumor. Certain apparent wide angles prove on careful examination to be due to the existence of double spindles (Fig. 7).

So far as can be determined from the limited number of cases examined, the different tumors tend to run true to type. It is true that in Case 17561, a carcinoma simplex in which only twelve angles, that is, six mitotic figures, were found suitable for measurement, I found a definitely narrow angle, $71^\circ \pm 11.6$, as compared with the other

* Slides used for examination of Ewing's tumor are in the collection of the Registry of Bone Sarcoma, Case UA 32-4, Dr. C. L. Connor, University of California Medical School.

carcinomas; yet even this is a wider mean angle than that found in most of the sarcomas. When we group together all the cases of a given type of tumor we find a difference in the mean values, as may be seen

TABLE II
Results of Measurement of Mitotic Angles

Case No.	Diagnosis	No. of angles measured	Result of measurement	
			Mean	Standard deviation
10753	Epidermoid carcinoma, Grade II	122	92°	±9.7
10526	Carcinoma simplex	40	88°	±5.4
10620	Carcinoma simplex	66	96°	±9.7
17561	Carcinoma simplex	12	71°	±11.6
10519	Carcinoma simplex	56	91°	±2.3
11968	Adenocarcinoma	30	87°	±8.1
117	Glioblastoma	110	61°	±8.4
18204	Melanotic sarcoma	102	56°	±9.6
30-1509	Fibrosarcoma	26 { 14 12	66°	±8.2
			114°	±5.7
32-1917	Fibrosarcoma	24 { 18 6	66°	±13.3
			111°	±6.7
32-2570	Fibrosarcoma	16 { 12 4	70°	±8.2
			101°	±3.8
32-2064	Fibrosarcoma, approaching neurogenic	66 { 20 46	78°	±13.1
			107°	±8.8
R 1362	Osteogenic sarcoma	20	65°	±10.5
33-239	Osteogenic sarcoma	24	73°	±13.5
R 1314	Ewing's tumor	12	88°	±10.7

from Table III. From these values one may conclude that the determination of the mitotic angle is a factor of value in differentiating types of tumors. The difference is particularly clear-cut, provided a fair number of angles are measured, between carcinoma on the one hand and sarcoma on the other. The cell of Ewing's endothelioma,

in the one case studied, has nearly the same angle as the epithelial cell; the spindle is very small as compared with the equatorial plate (Fig. 4).

As stated above, the fibrosarcomas that we have measured thus far show two distinct types of mitosis, the narrow and the wide spindle. As will be noted later, the wide spindle may prove, at least in some instances, to be actually two partly superposed spindles, really a masked multiple mitosis. The osteogenic sarcoma varies distinctly from the fibrosarcoma in exhibiting only one type of mitotic figure,

TABLE III

*Values of Mitotic Angles for Different Classes of Tumors **

Diagnosis	No. of angles measured	Result of measurement	
		Mean	Standard deviation
Carcinoma	346	91°	± 10.1
Glioblastoma	110	61°	± 8.4
Melanotic sarcoma	102	56°	± 9.6
Fibrosarcoma	144 { 70 74	72° 108°	± 12.6 ± 8.4
Osteogenic sarcoma	44	69°	± 12.8
Ewing's tumor	12	88°	± 10.7

* Includes measurements not presented in Table II.

a relatively narrow one, the mean being $69^{\circ} \pm 12.8$. This is closely approximated by the one case of glioblastoma that we have been able to examine, which showed a mean angle of $61^{\circ} \pm 8.4$ (Fig. 3). There is, of course, not a significant difference between these two figures obtained. However, the spindles of this glioblastoma are distinctly rounded off toward the centrioles, in contrast to the very sharp angles of the sarcomas.

The one case of melanotic sarcoma that we have been able to examine showed a comparatively narrow angle, $52^{\circ} \pm 10.3$ (Fig. 6). This is the narrowest of the mitotic angles examined, except that of the lymphoblast. I have not yet available a sufficiently large number of mitotic angles to report definitely on the lymphoblast. Pre-

liminary measurements correspond fairly closely with those of Ellermann² and of Petri,³ about 40°, although a few run as high as 50° (Fig. 1).

Another type of tumor of which we have too little material on which to base an opinion is the Ewing's endothelial myeloma. Examination of one case showed a mean angle of 88°±10.7. This, however, is based on only twelve determinations. The goniometer

TABLE IV
Readings Obtained in Measurement of Mitotic Angles of Carcinoma Simplex

Case No. 10620					
85	86	94	97	90	86
100	105	109	97	102	93
90	91	118	100	90	117
118	110	104	100	116	90
90	90	120	86	98	95
98	97	95	91	90	101
119	112	94	88	93	98
80	87	102	107	93	85
77	83	89	87	96	94
97	99	83	99	90	92
93	104	93	99	96	98
Mean, 96°.		Standard deviation, ± 9.7 .			
Case No. 10526					
89	85	83	87	99	91
90	87	85	79	88	90
90	101	90	81	98	94
90	90	92	85	95	88
85	87	79	84	85	92
81	82	90	92	86	94
90	83	78	80		
Mean, 88°.		Standard deviation, ± 5.4 .			

could not be successfully used in this case because of the small size of the spindle, and enlarged photomicrographs were used.

In order that some idea of the variation encountered in the values obtained for the angles may be given, in addition to the information provided through the standard deviations, I am including Table IV, which gives the direct reading obtained with the ocular goniometer in typical examples of carcinoma simplex, No. 10620, with a fairly high standard deviation, and No. 10526, with a small standard deviation.

One point emphasized by these measurements is the not infrequent occurrence of greater degrees of inequality in the angles of the

two ends of the spindle than can be accounted for by errors in measurement. Whereas in the normal cell apparently a symmetrical spindle is the rule, in the cells of malignant tumors varying degrees of asymmetry are not infrequent. These variations at times are apparent rather than real, as with careful inspection two sets of centrosomes with two overlapping spindles may be distinguished. At times, particularly where angles are apparently very wide, there may be actually two distinct spindles side by side (Fig. 7). In the early stage of the formation of a triaster it is possible to have two more or less closely located centrosomes on one side of the equatorial plate and a single centrosome on the other side. This type of mitosis is always either clearly distinguished or else is hinted at by the irregularity of arrangement of the chromosomes, or a definite branching of the equatorial plate (Fig. 8).

That the angle is to some extent independent of the shape of the cell is well indicated by Figures 1 and 4, in both of which the cells are loosely arranged and similarly shaped, and the angles very different. However, the narrow angles obtained in the epithelial tumor No. 17561 occurred in a closely packed focus of spindle cells, and in spindle-celled portions of sarcomas also the angles tend to be narrow, apparently influenced somewhat by the shape of the cell.

The outer linin fibrils of the spindle usually fail to reach the outer portion of the equatorial plate, as is brought out in all the figures. In certain instances the linin fibrils are bowed outward, as though no stress were exerted on them. No explanation for this has yet been found. In the sarcomas studied the axis of the spindle is parallel to the long axis of the cell, while in carcinomas the axis of the spindle is usually independent of the axis of the cell.

SUMMARY AND CONCLUSIONS

1. On the basis of the cases thus far studied the angle of the mitotic spindle provides a method by which carcinoma can be distinguished from sarcoma and from lymphoblastoma.
2. Because of the two types of mitosis apparently usually occurring in the same tissue in fibrosarcoma, as distinct from other types of sarcoma, this particular form may be distinguished.
3. The mean values obtained for the mitotic angles in carcinoma are 91° , in melanotic sarcoma 56° , in fibrosarcoma 72° for the narrow

spindles and 108° for the wide spindles, in osteogenic sarcoma 69° , and in Ewing's tumor 88° .

4. Further study of other types of tumor and normal cells is now in progress.

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DESCRIPTION OF PLATES

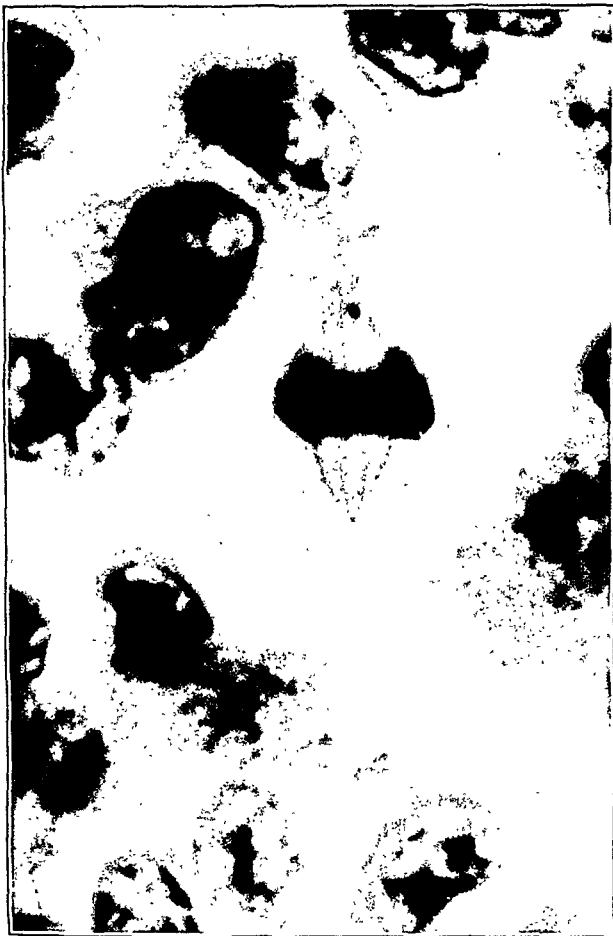
PLATE 126

FIG. 1. Case No. 18204. Mitotic spindle in normal lymphoblast from germinal center. Phosphotungstic acid hematoxylin stain. $\times 2350$.

FIG. 2. Case No. A 27-4. Epidermoid carcinoma, Grade 2. Mitotic spindle in tumor cell. Phosphotungstic acid hematoxylin stain. $\times 2350$.

FIG. 3. Case No. 117. Glioblastoma. Mitotic spindle in tumor cell. Hematoxylin and eosin stain. $\times 1500$.

FIG. 4. Case No. UA 32-4. Ewing's tumor (endothelial myeloma). Mitotic spindle in tumor cell. Hematoxylin and eosin stain. $\times 2350$.



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Warren

Angle of Mitotic Spindles in Malignant Cells

PLATE 127

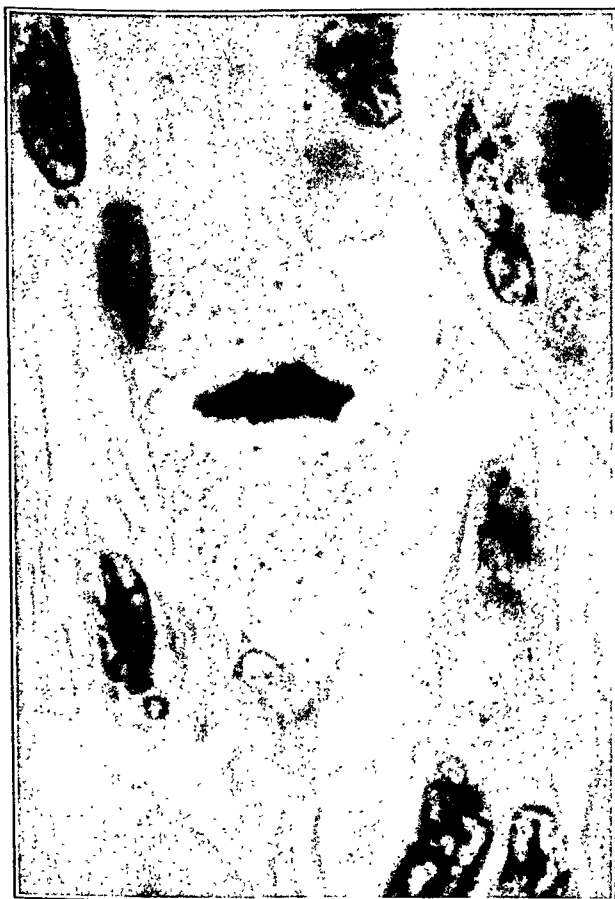
- FIG. 5. Case No. 11966. Fibrosarcoma. Mitotic spindle (narrow type) in tumor cell. Phosphotungstic acid hematoxylin stain. $\times 1500$.
- FIG. 6. Case No. 18204. Melanotic sarcoma. Mitotic spindle in tumor cell. Phosphotungstic acid hematoxylin stain. $\times 2350$.
- FIG. 7. Case No. 32-2064. Fibrosarcoma. Double mitotic spindle in tumor cell. Phosphotungstic acid hematoxylin stain. $\times 1500$.
- FIG. 8. Case No. 18204. Melanotic sarcoma. Triaster. Phosphotungstic acid hematoxylin stain. $\times 2350$.



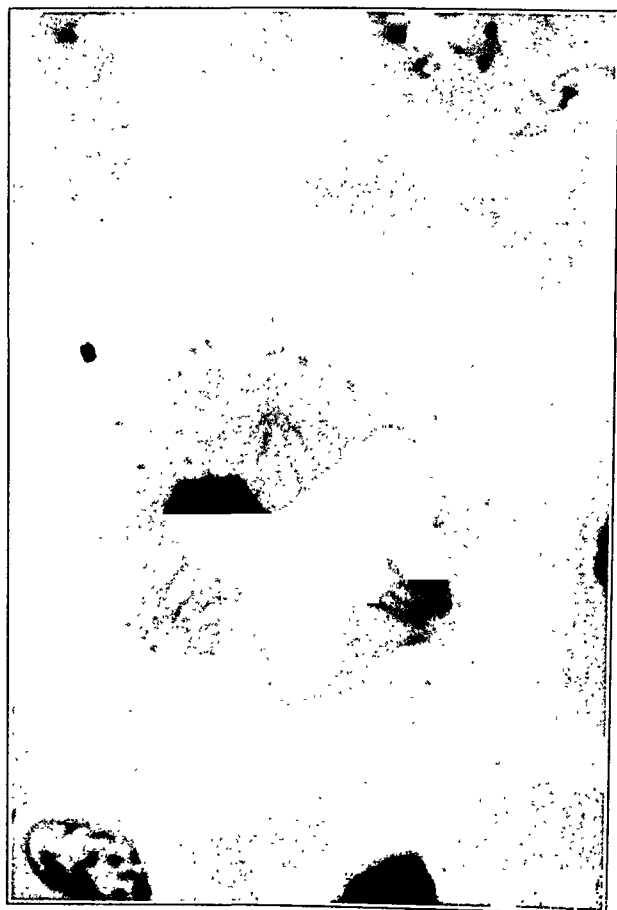
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THE EFFECT OF CAESIUM CHLORIDE ON TRANSPLANTED TUMORS OF MICE *

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Numerous experiments have been carried out to determine the effect of various chemical elements on the growth of malignant tumors in animals and in man. Of these elements lead,¹⁻³ thallium,^{4, 5} rare elements such as thorium, yttrium, uranium, and so on,⁶⁻⁹ and strontium, cadmium and barium,¹⁰ have received special attention, and valuable information concerning their action on growing neoplasms has been accumulated. As far as is known, however, caesium has been studied only casually in this connection,^{7, 11, 12} and the writers have been observing the effect of this element on the growth of transplanted malignant tumors of mice. In the present paper a preliminary report of some of these studies is made.

Caesium is the fifth element in the alkali series and is the most strongly basic of them all, with the exception of element 87, which is not yet available for study. Administered as the chloride, caesium is only moderately toxic for animals. It is excreted quite rapidly, chiefly in the urine but also to a less degree in the feces. Traces of the element, however, may be found in the animal body up to about 9 days after the last injection of the salt.

In the experiments here described caesium chloride, C. P., was the salt uniformly employed. An aqueous solution of this salt containing 25.91 gm. per liter is isotonic with physiological (0.9 per cent) sodium chloride solution, and this concentration was chosen as standard. Subcutaneous injections of small amounts of this solution into mice produced considerable immediate local irritation and the animals were nervous and unusually lively for 5 to 10 minutes after the administration. Previous studies¹³ of the physiological effect of caesium chloride on normal mice had shown that 0.2 cc. of the 2.591 per cent solution was the maximum daily dose the mice would tolerate, and 0.1 or 0.2 cc. quantities were usually employed in the experiments. Injections were always made into the subcu-

* Received for publication June 19, 1933.

taneous areolar tissue of the back, well away from the tumor if one were present.

For the experiments the Twort carcinoma was employed. This tumor originated as a carcinoma of the mammary gland of a mouse. It was found to be readily transmissible to other mice if a small fragment of the tumor tissue were implanted subcutaneously. The take rate is somewhat variable but in our experience approximately 80 per cent of the tumors were visibly growing 10 to 20 days after implantation. The tumor is characterized by a tendency to grow quite rapidly, to metastasize to the lungs at a fairly early period, and to undergo early spontaneous necrosis in its central portion. At 8 weeks it reaches moderately large size, averaging about 3 cc. in volume. Metastasis to the lungs has usually occurred at 12 weeks, often earlier.

Histologically the Twort carcinoma is a rather rapidly growing neoplasm obviously of epithelial origin. The tumor cells at the peripheral portions of the growth, where they are viable, are elongated or spindle-shaped, sometimes polyhedral, usually poorly differentiated. They contain large, irregularly oval, often lobulated nuclei in which small numbers of minute, pale-staining chromatin granules are present. The cytoplasm is moderately abundant, faintly acidophilic, and occasionally vacuolated. Large, pale, hyaline nucleoli, from one to five per nucleus, are usually observed. Mitotic figures are abundant. In general the neoplastic cells grow in irregular sheets or cord-like masses without definite gland formation but with a distinct tendency to the production of alveolar structures. The stroma consists of delicate strands of connective tissue in which thin-walled blood vessels are present but not abundant. The major portion of the tumor, beginning in the center, becomes necrotic at an early period and most of the sections show extensive foci of degenerated tumor tissue in which perithelially arranged, small groups of viable cells may often be found. Because the tumor cells are large and rapidly growing the neoplasm is ideal for the study of the effect of an agent that may act primarily on the growing cell.

The white mice used in the experiments were obtained from one dealer and were said to be of the same strain. Nothing is known concerning the racial or hereditary characteristics of the animals, but they were found to be quite healthy and free from disease and

spontaneous tumor development. In general, male mice weighing from 20 to 25 gm. each were employed in all of the experiments.

The technique of implanting the tumor into the mice was as follows. The host mouse was killed with ether and the tumor removed aseptically and placed in a sterile, covered Petri dish. Small blocks of the tissue were preserved in Zenker's fluid or 5 per cent formaldehyde solution for future histological study. All recognizable necrotic material was separated and discarded. From the remaining viable neoplastic tissue small fragments measuring about 0.2 cm. in diameter were prepared. The skin of the mouse to receive the implant was sterilized with alcohol, nicked with sharp scissors, the subcutaneous areolar tissue stretched and a fragment of tumor tissue introduced. The wound edges were drawn together and sealed with collodion and the animal returned to the cage. At first each mouse was anesthetized for the procedure, but subsequent implantation without anesthesia was found to be readily tolerated. Of several hundred mice implanted in this fashion not one developed postoperative infection. Mortality, however, was somewhat higher in the anesthetized mice than in those in which no anesthetic was employed.

EFFECT OF CAESIUM CHLORIDE, ADMINISTERED AFTER TUMOR IMPLANTATION, ON THE GROWTH OF THE TWORT CARCINOMA

Forty-two healthy white mice, all males from the same breeder's lot, were inoculated on May 6th with Twort carcinoma from a mouse that had received the tumor 86 days previously. After implantation the mice were divided into four groups, one of which was kept as a control. The animals in the other groups were given daily subcutaneous injections of 0.2 cc. of a 2.591 per cent solution of caesium chloride, beginning 1, 10 and 20 days, respectively, after implantation.

At the end of 10 days 8 of the control mice showed small but apparently growing tumors, while 2 had none. The mice of the first group injected with caesium chloride solution had suffered high mortality, 8 of the mice having died, apparently from an epizootic. Of the 2 that remained 1 had a growing tumor, the other had none.

In the other two groups the tumor take rate was approximately that of the control series: 9 alive in one group — 8 with small tumors

and 1 with none; 8 alive in the other — 6 with small tumors and 2 with none.

At this time daily caesium chloride injections were begun in the group in which 9 mice were alive. At the end of 10 days 5 were living, all with definite tumors that continued to grow in spite of continued caesium chloride injections.

Twenty days after implantation the last group, now reduced to 6 animals, 4 of which had definite tumors while 2 had none, were started on daily doses of caesium chloride. At the end of a month of treatment one of the four tumors had regressed but the others had grown considerably. The 2 mice without tumors continued to be tumor-free.

The mice of all groups except the controls continued to receive daily injections of the caesium chloride solution. The tumors grew progressively and one by one the animals died, those that had received caesium as well as the controls. Once the tumors appeared caesium chloride had no evident effect, except in a single case where an apparently growing neoplasm regressed after caesium chloride injections were started. It cannot be said that in this case the regression was due definitely to the effect of caesium, since spontaneous regressions are known to occur.

EFFECT OF CAESIUM CHLORIDE ON THE LARGE, GROWING TWORT CARCINOMA

Since caesium chloride seemed to have no great effect upon the growth of the Twort carcinoma when injections were begun soon after implantation, it was decided to study the effect of the salt upon large, well developed tumors. A group of mice had been implanted with tumors on February 10th for another experiment. By April 26th approximately 80 per cent of the control animals in this series had large tumors. Twenty of these mice with well developed tumor masses were divided into two equal groups, one of which was kept as a control.

Daily injections of 0.2 cc. of the caesium chloride solution were begun on the second group, with the result that none of the injected animals became tumor-free. In several cases, however, the neoplastic tissue seemed to be softer and more extensively degenerated

in these animals than in the controls. However, death, either from the neoplasm itself or from some associated secondary cause, occurred in all cases, the first mouse dying on May 14th, the last on July 22nd. The control mice likewise showed no tendency to tumor regression and death occurred on various days between May 12th and July 2nd.

Careful gross examination of the tumors from the animals that had received caesium chloride showed large, soft, necrotic, cystic masses, the centers of which were broken down and liquefied. As usual the only viable tissue, if present, was found at the periphery of the nodules. In some cases little if any living tissue could be detected grossly. Metastases, however, were generally present in the lungs, so that caesium chloride injections had had no deterrent effect upon the formation and growth of metastases.

The tumors from the control mice were similar in most respects to those of the treated animals, except that necrosis appeared less generalized and viable tissue was uniformly found. Pulmonary metastases were present in practically every animal.

Histologically the neoplastic tissue from both experimental and control animals differed but little, except for the fact that, as was noted grossly, necrosis of the tumors was sometimes more extensive in the treated animals. But whether or not this effect was due to caesium it is impossible to say. The caesium salt was thus found to have no perceptible effect on the size of the growing Twort carcinoma, and doubtful, if any, effect upon its gross and histological structure.

EFFECT OF PRELIMINARY INJECTIONS OF CAESIUM CHLORIDE ON THE TAKE RATE AND GROWTH OF THE TWORT CARCINOMA

Forty healthy, male, white mice from the same breeder's lot were divided into two equal batches which were caged separately. To one lot daily subcutaneous injections of 0.2 cc. of the standard caesium chloride solution were given for a period of 9 days. For some unexplained reason, possibly caesium poisoning, 8 of the treated mice died during this time. On the 10th day the living experimental animals and 12 of the controls were implanted with the Twort carcinoma which came from a mouse that had received the tumor 63 days previously.

The experimental mice were then continued on daily injections of caesium chloride, a smaller amount, 0.1 cc., being employed. On the 18th day 5 of the treated mice had definite tumors (41.6 per cent) and 7 (58.4 per cent) had none, while 10 of the controls (83.3 per cent) had growing tumors and only 2 (16.7 per cent) had none. On the 36th day the findings in the control group were the same. Of the 5 treated mice with tumors, however, 2 had become practically tumor-free.

The tumors in the control mice were large, elastic and rather soft to the touch. On removal from the body they were smooth-surfaced, slightly lobulated and friable. On section the cut surfaces of the viable portions were pinkish with a pearly luster, and the entire mass resembled the typical Twort carcinoma described above, with soft, necrotic, cystic centers. There was considerable evidence of invasion of the abdominal wall, but no peritoneal or pulmonary metastases were found at this stage of growth.

Histologically the tumors were typical carcinomas of the type described above. Foci of necrosis were everywhere visible. In the viable portions the tumor cells were large, undifferentiated, and rapidly growing. Mitotic figures were abundant. A rough average of eight to nine per field was determined by counting at random a total of thirty oil immersion fields in sections from 6 of the animals.

The tumors in the treated mice were smaller, firmer, and more solid than were those in the controls. Grossly they were smooth-surfaced, finely nodular, pinkish, and friable, although less friable than the tumors in the control animals. On section, however, there was little to differentiate these tumors from those of the untreated mice. Viable tissue was peripherally placed and central necrosis of the mass was no more marked than in the animals described above. Metastases to the lungs and extension into the peritoneal cavity were not present.

From a histological standpoint the tumors resembled those of the untreated controls, consisting of a peripheral zone of moderately large, undifferentiated, polyhedral or elongated epithelial cells growing in alveolar masses and surrounded by delicate, slightly vascular, fibrous stroma. Rarely there was a tendency to gland formation. Widespread foci of necrosis were present, but neither the extent nor the character of the necrosis differed from that noted above. The cytoplasm of the tumor cells was somewhat more basophilic than

that of the control tumors, but it was not appreciably diminished in amount. Cell nuclei likewise showed no morphological differences. Nucleoli were present and mitotic figures were relatively abundant. In thirty oil immersion fields taken at random an average of seven to eight mitoses per field was found. Thus in spite of gross differences between the tumors of control and treated animals no histological differences of note could be detected.

Sections taken from the subcutaneous tissues of the animals, both experimental and control, in which tumors did not appear, showed little of note histologically. At 10 days necrotic tumor implants, surrounded by an inflammatory reaction, were found. At later periods no tumor tissue at all could be discovered.

A second lot of 34 male white mice was divided into two groups, to one of which daily subcutaneous injections of 0.1 cc. of a 2.591 per cent solution of caesium chloride were given for 4 days, after which the dose was increased to 0.2 cc. for 9 days. The Twort carcinoma was then implanted into all the mice, both experimental and control. After implantation daily injections of 0.2 cc. caesium chloride were given to the experimental mice.

At the end of 36 days 4 of the experimental mice were dead. Of the living animals 6 (46.2 per cent) had no tumors, while in 7 (53.8 per cent) small ones were present. Of the control mice 4 had died. Eleven (84.6 per cent) of the survivors had large tumors while 2 (15.4 per cent) had none.

The size of the tumors in the treated and untreated groups was significant. The average diameter of the tumors in the injected mice was 0.9 cm.; the total volume of tumor tissue in all the animals together was 3.3 cc. The average diameter of the tumors in the control animals was 1.25 cm.; the total volume of all tumor tissue was 14.7 cc. There was thus a striking difference between the actual amount of tumor tissue that grew in the two sets of animals.

Grossly the tumors from the control mice were fairly large, a few measuring 2 to 2.5 cm. in diameter. They were soft, friable, lobulated, necrotic and often superficially ulcerated. Their centers were liquefied. Histologically the viable portions consisted of rapidly growing cells similar to those described above. An average mitotic figure count of seven to eight per field was determined from a study of thirty oil immersion fields. Considerable necrotic tissue was present.

The tumors of the injected animals were much smaller than those of the controls. They were firmer to the touch and seemed more solid but on section their centers, like those of the control tumors, were broken down and liquefied. Histologically there was little to differentiate the two types of tumors, treated and untreated. The neoplastic cells of the treated tumors appeared more densely packed together and were slightly more basophilic than those of the controls. In thirty oil immersion fields an average of seven to eight mitoses per field was found. Active cell division was thus as abundant in the treated tumors as in the untreated.

Caesium chloride injections, begun 9 to 13 days before tumor implantation and continued thereafter, apparently diminish the take rate of the Twort carcinoma and result in the growth of tumors which are grossly considerably smaller and more compact than those of control animals, but which show practically no histological differences when the tumor tissues are studied by the usual tinctorial methods. There is little evidence that the tumor, once actively growing in the animal body, is perceptibly affected by the administration of the caesium salt.

DISCUSSION

In considering the activity of the cell nucleus in rapidly growing normal or neoplastic tissues the question arose as to the effect a strongly basic ion might exert on nuclear activity, if it were introduced into the reaction system. One may conceive of the cell nucleus as containing, among other substances, a readily oxidizable sugar, phosphoric acid, certain nitrogenous compounds, and various basic ions, sodium, potassium, calcium, and so on. Some of these substances, according to Levene and Bass,¹⁴ are capable of combining to form the compound that is peculiar to every cell nucleus, namely, nucleic acid. This complex substance, in its simplest form, Levene believes to be "an ester of phosphoric acid and an organic radicle, the latter consisting of a sugar and a nitrogenous component which is a cyclic derivative of urea." In the opinion of this investigator animal nucleic acid (zoönucleic acid) is a tetranucleotide consisting of four molecules of phosphoric acid attached to four molecules of a reduced pentose, namely *d*-ribodesose, to which in turn are linked four nitrogenous compounds, adenine, guanine, cytosine and thymine.

In any given cell nucleus the general proportions of the constituents of nucleic acid, in solution and ready to combine, are probably fairly constant. So, too, are the various basic ions. The product of the reaction system, phosphoric acid-pentose-basic ion, is therefore pentose-phosphoric acid ester and basic phosphate, presumably in a fairly constant ratio. However, if one or the other of the various reacting substances were to be altered or replaced, there should theoretically be some effect upon the activity of the system. If, for example, the unstable pentose, *d*-ribodeseose, were to be increased, we might well conceive of possible resultant increased activity of the cell nucleus because more nucleic acid, so readily capable of hydrolysis and oxidation, would tend to be formed.

If, on the other hand, a powerful basic ion were to be introduced into the system we might conceive then of the formation of increased amounts of basic phosphate at the expense of the nucleic acid. This would theoretically result in the formation of a relatively inert substance at the expense of an unstable compound capable of marked chemical activity. It is not unreasonable to conclude, therefore, that a nucleus containing such a powerful basic ion would be less active than usual. If such a condition could be produced in rapidly growing malignant cells the result might be interesting.

To test the validity of such an hypothesis the experiments outlined above were devised and the element caesium, the most strongly alkaline and electropositive of all of the available alkali elements, was chosen as the actively basic ion to be introduced into the reaction system. So far the results have been disappointing. Caesium chloride, the salt of caesium that was uniformly employed, injected soon after the implantation of the Twort carcinoma into mice, has no appreciable effect upon the growth of the tumor. If daily injections be started after the tumor has reached a relatively large size the salt has no effect whatever, except possibly that of increasing the amount of necrosis occurring in the tumor, yet even this is uncertain.

If, however, the tumor be implanted into mice already saturated with caesium, and which continue to receive daily injections of the caesium salt, there is evidence of some deterrent effect of the element upon the growth of the neoplasm. In the first place, the percentage of takes is less in the caesium-injected mice than in the controls, 41.6 per cent in one series and 53.8 per cent in another.

The control mice in these series showed a much higher percentage of takes, 83.3 per cent and 84.6 per cent respectively. It is thus possible that the tumor fragment, introduced into a tissue medium in which highly alkaline ions are supposedly present, is unable to establish itself readily as a growing implant. In the second place, the individual tumors, if they do appear in the treated animals, are smaller and somewhat more firm in consistence than the control growths. However, they exhibit practically no tendency to regress once they have developed, and histologically they show no readily recognizable structural changes that would differentiate them from the controls.

Caesium, if present in the animal body before tumor implantation, thus exhibits an apparent deterrent effect upon the take rate and the subsequent growth of the Twort carcinoma, as indicated by the clinical character of the growth. Morphologically, however, no significant changes can be found in the tumor cells themselves. The probability is that the effect produced by caesium is essentially chemical in character, with no recognizable alteration of the cellular structure. From careful cytological study of the transplanted tumor tissue in this small series of caesium-injected animals it is impossible to discover any obvious diminished proliferative activity on the part of the tumor cells. These cells are apparently as abundant, as healthy and as active in the tumors of treated mice as they are in the neoplasms of the control animals.

SUMMARY AND CONCLUSIONS

The theoretical hypothesis that the introduction of strongly basic ions into the nuclear complex might greatly reduce nuclear activity is not supported by the study of the effect of caesium upon the growth of the Twort carcinoma of mice. There is no evidence in these experiments that the presence of the element caesium in the body of a mouse with established Twort carcinoma affects in any way the capacity of the tumor cells to divide and multiply. There is, however, suggestive evidence to indicate that necrosis of the neoplastic tissue may be more extensive in the caesium-treated animals. That caesium under certain conditions has a deterrent effect on tumor growth is indicated, and further studies of this property of the element are in progress.

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THE DIAGNOSIS OF TUMORS BY ASPIRATION *

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During the past three years the author has studied smears of material from some 2500 tumors obtained by aspiration with an 18 gauge needle. The method of securing the tissue has been described by Martin and Ellis,¹ but since the actual technique is of considerable importance it may be described again in brief. The needle used is an ordinary 18 gauge needle attached to a Record syringe. The skin is infiltrated by a drop of novocaine and a very small puncture wound is made with a bistoury to avoid contamination of the tissue to be aspirated with surface epithelium. The needle is introduced with the piston *closed* until it is felt to enter the neoplasm. Then the piston is partially withdrawn, thus creating a negative pressure within the syringe, and the needle is advanced into the tumor. Portions of tumor are forced by the negative pressure into the needle. The manoeuver is usually repeated once without withdrawing the needle from the mass. When the needle is withdrawn care is taken to release the piston gradually, in order to prevent a sudden spraying of the contents of the needle over the walls of the syringe. Material obtained is placed on a slide and smeared out by pressure with another slide. Gentle heat is applied and the smear is dropped into alcohol for a minute or two, after which it is stained with hematoxylin and eosin, dehydrated, cleared and mounted like any section. If much fluid is obtained small fragments of tissue are selected with a wire loop and smeared on slides, or, if sufficiently large, they are sometimes sectioned. Blood clots may be sectioned in the same manner as tissue.

It is the purpose of this paper to discuss the usefulness of this method in diagnosing tumors and to call attention to certain pitfalls and limitations. In the Memorial Hospital laboratory over 6000 pathological specimens are received annually for diagnosis. Many of these are small biopsies. It is not the practice of the clinicians to use the method of aspiration when the ordinary biopsy can be obtained from an ulcerating surface, but under many circumstances

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where pathological diagnosis is required it is impossible or undesirable to attempt the usual form of biopsy. Furthermore, certain therapeutic procedures employed in this hospital make it essential, or at least simpler for the patient, to depend frequently on aspiration for diagnosis when others would secure a surgical biopsy.

Material obtained by aspiration must necessarily consist of the more unattached constituents of a tissue, cells that are readily displaced into the needle by a little manipulation. Hence, most smears of such tissues consist of loosely arranged sheets of cells or, in the more cellular tumors, of showers of isolated cells or clusters of a few elements. Since the smears are subjected to but little fixation the tumor cells appear as a rule larger than in fixed tissues. For the most part the coarser morphology of the tumor is lacking, the relations of parenchyma to stroma often cannot be determined and in interpreting the smear one must relearn by experience the characteristics of the various tumors. To do this it will aid greatly if one aspirates gross tumors coming to the laboratory for the usual type of diagnosis, thus confirming by sections the pictures shown in smears. Before interpreting an aspiration it is the writer's custom to be familiar with the clinical history of the case and the exact location of the suspected mass, together with the clinician's impression of the diagnosis. The pathologist should know from the clinical description what types of tumors may present themselves in a given clinical setting. Only then is it possible to put together the various minutiae of a smear and render the probable diagnosis. It may be well to recite briefly a case in point.

The patient was a middle-aged woman who entered the clinic of the Memorial Hospital complaining of a recurrent subcutaneous mass in the scalp. She presented a hard, lobulated, movable, subcutaneous bulging tumor over the vertex. No source of metastasis was found. Aspiration yielded pure cartilage (Fig. 1). The obvious diagnosis was chondroma, yet the location of the tumor was scarcely consistent with that diagnosis; the tumor was movable on the deeper structures and radiographs of the skull revealed no bone defect. It was therefore necessary to consider in the diagnosis some type of tumor that could occur in the scalp without relation to bone, and that could yield cartilage on aspiration. The most obvious suggestion was, of course, a tumor of cutaneous glands — either sweat glands or sebaceous glands — with extensive cartilaginous metaplasia, such as occurs in salivary gland tumors. Later examination of the tumor after surgical removal proved it to be an adenocarcinoma of the sweat glands with marked cartilaginous metaplasia, thus confirming a diagnosis rendered upon a combination of data derived from aspiration and from clinical experience. Neither alone could have sufficed.

Under most circumstances it has not been necessary for us to determine from aspirated material the exact histological subvariety of tumor. The usual question involved is merely the presence or absence of a malignant tumor, since the clinician, when informed that a lesion contains cancer cells, is usually quite capable of determining from other data the essential type of disease. Merely ascertaining the presence of cancer is much simpler than determining its particular variety. The latter is oftentimes impossible from smears.

Conservative surgeons or radiologists may be appalled at the idea of introducing a needle through sound tissue into a cancerous mass. The avoidance of trauma to a malignant tumor is doubtless highly desirable, yet quite impossible if one is to secure a histological diagnosis, and the burden of proof that surgical excision is a less traumatizing procedure than simple needle aspiration appears to the writer to rest on those who advocate the former method. Suffice it to state that at the Memorial Hospital no untoward result can as yet be attributed to needling a tumor, whereas unfavorable consequences have been observed to follow surgical biopsies, particularly when performed by surgeons unfamiliar with the hazards of biopsy in certain types of tumors, especially those of bone.

It was emphasized at the beginning of this paper that biopsy by aspiration is not performed in ulcerating lesions where a small bit of tissue is readily secured with the biopsy forceps. In any large tumor clinic, however, it is necessary for various reasons to secure specimens from deep seated lesions where an ulcerated surface is not accessible.

The remainder of this brief paper is best devoted to a discussion of various types of tumors where aspiration is of practical value. Prominent among such lesions is the possibly cancerous cervical node. In many surgical clinics a routine neck dissection is performed on all patients with intra-oral carcinoma when the disease is still operable. At the Memorial Hospital the tendency in recent years has been to limit more and more this rather drastic procedure. At the present time very few neck dissections are done. Since for the most part intra-oral carcinomas metastasize to but one or two nodes, usually situated at the carotid bulb, it is the practice to radiate the neck externally and, later on, in the case of the more radioresistant lesions, to expose the nodes surgically and to implant an adequate amount of gold radon seeds in persistent node metastases. The ultimate out-

come of this procedure, as judged by five year statistics, is still in doubt and it may be abandoned or greatly modified. Since, however, the results of surgical treatment of established metastatic carcinoma of the cervical lymph nodes are rather unfavorable, and since many patients present an inoperable or doubtfully operable setting, an experiment with a more conservative method of therapy is fully justified. Moreover, in the case of many types of intra-oral carcinoma—notably the transitional cell carcinomas and lympho-epitheliomas of the tonsil, base of the tongue, soft palate, epiglottis, arytenoid cartilages and pyriform sinuses—the metastatic foci are often far beyond the hope of even palliation by neck dissection. Since for statistical purposes, as well as for determining the immediate therapeutic procedure, it is desirable to have histological proof that an enlarged cervical node contains metastatic tumor, every suspected node is subjected to aspiration. We have examined 725 such aspirations and find it for the most part simple to distinguish cancer cells. The large sheets of flattened, spindle or polyhedral cells, with central masses of pink-staining cells more squamous in shape, stand out in sharp relief from the typical lymphocytes of the node. The cells of transitional cell carcinoma are smaller, usually more spindle in contour and lack squamous characteristics, unless the node has been subjected to radiation. Oftentimes a metastatic node undergoes central liquefaction and under these circumstances only masses of débris and unattached pink-staining, rather hyaline squamous cells may appear in the smear. Calcification may occur. Reaction to necrotic squamous pearls leads to the appearance of foreign body giant cells, which may give rise to confusion with tuberculosis. The history of the case is of decisive significance in this diagnosis. Much this same appearance may arise in smears from the contents of a branchiogenetic cyst. In fact the cyst, with its sloughing epithelium, cellular detritus, calcified material and lymphocytes may duplicate the necrotic metastasis of squamous carcinoma. Again, the history of a prior intra-oral carcinoma makes the diagnosis. When no primary tumor is found repeated aspiration may be necessary for diagnosis. Sooner or later, clusters of typical tumor cells will appear in fluid aspirated from the metastatic deposit, provided the extent of collapse of the cyst or its rate of refilling does not clear up the diagnosis before repeated aspiration is necessary.

Aspiration finds another field of usefulness in the diagnosis of

lesions of the antrum. In their earlier stages, when they present no ulcerating surface either in the nose or palate and when radiographs show no bone destruction permitting radiographic diagnosis, the aspirating needle is regularly used to distinguish antral carcinoma from either empyema or polyposis. Our records contain data on 50 aspirations of antral lesions. In some cases it has been impossible to return any diagnosis other than carcinoma. In many instances it has been possible to distinguish by their finer characteristics such specific types as epidermoid carcinoma, Schneiderian carcinoma by its mixture of mucus-containing cells, salivary gland tumors by their small cells, cystic adenoid features, and mixture of mucoid, spindle cell areas. In one instance it was possible to differentiate carcinoma from osteogenic sarcoma, thus relieving a patient from the distressing consequences of a surgical and radiological assault designed for the cure of carcinoma of the antrum. In the case of this patient a surgical biopsy would have subjected an osteogenic sarcoma to the well known consequences of incision through an infected field, whereas the aspiration was tolerated perfectly.

The aspiration of centrally located, destructive lesions of the jaw bones is often useful. The radiologist is at times at a loss to distinguish the simple cyst from either adamantinoma or giant cell tumor. The clinician at times hesitates between the diagnosis of extension of oral cancer to the bone or osteomyelitis. Our laboratory contains records of 57 aspirations of the jaw bones, mostly of the mandible. The simple cyst yields as a rule a considerable quantity of straw-colored fluid and on centrifuging one usually recovers scattered, flat, pink-staining polyhedral cells, leukocytes and crystalline material. The giant cell tumor is readily recognized by the large numbers of typical epulis giant cells. Adamantinoma yields in its typical form bits of spindle cell tissue surrounded by columnar basal cells. Very few adamantinomas have occurred since aspiration was instituted and we cannot say that their different degrees of aggressiveness can be recognized from smears. The cystic adamantinoma may present a wall lined by tissue resembling the *débris paradentaire* of Malassez. It is essentially an adamantinoma, yet it may lack features that distinguish it in smears from more aggressive tumors of similar origin.

The parotid tumors are peculiarly suited to aspiration. At the Memorial Hospital it is the practice to excise the freely movable

mixed tumor and to depend on radiation in various forms for the treatment of the inoperable mixed tumor or the various types of true malignant carcinomas. Smears of mixed tumors (Fig. 2) show various combinations of cartilage, mucinous tissue and epithelium. Because these features are absent and the smear shows epithelium only, it does not necessarily mean that the tumor is a fully malignant carcinoma capable of metastasis, since the needle may reach but one portion of a tumor of quite diverse structure. The clinical features of the case are of great importance when the characteristics of the smear lead to doubt. In most instances no difficulty is encountered in recognizing the highly malignant tumor from smears (Fig. 3). We have studied 66 parotid aspirations.

When the primary lymphadenopathies, Hodgkin's disease, lymphosarcoma, pseudoleukemic lymphoma, Brill-Symmer's lymphadenoma and the like, are suspected from clinical examination, an aspiration is frequently performed to rule out the presence of metastatic tumor. With metastatic tumor eliminated it is preferred to submit the patient to a surgical biopsy rather than to depend on aspiration. It has been possible to make a tentative diagnosis of Hodgkin's disease from smears when the tissue showed a distinct eosinophilia or many Sternberg cells, yet for the most part it is our feeling that the fixed smear is unreliable. It will be remembered that Forkner² reported little difficulty in distinguishing various types of primary lymph node lesions by examining supravital stained preparations of bits of tissue obtained by inserting a dental broach through a trocar introduced into the node. We have not examined supravital preparations. We find oftentimes great difficulty in distinguishing or classifying primary lymph node lesions when large, excellently stained specimens are available for study. Hence, we are sceptical about the possibility of making such diagnoses from smears. Certain highly characteristic lymphadenopathies, such as Gaucher's disease, may be readily recognized. Spleens from cases of suspected Gaucher's disease have been aspirated on two occasions and the diagnosis subsequently confirmed by splenectomy.

Nearly 500 breast lesions have been subjected to aspiration. The indications for aspirating a breast tumor may be tabulated as follows:

1. For confirmation of diagnosis in patients who, because of something in their general physical status, are to be selected for treatment

by irradiation alone, and from whom it is not possible to secure tissue for examination unless the patient comes to autopsy.

2. For confirmation of diagnosis in young patients, or when tumors develop in the course of lactation or pregnancy, under which circumstances certain clinicians feel that radical surgery is not the method of choice in treatment on account of the usually unfavorable outcome of the case.

3. To rule out suspicious nodules in the opposite breast in patients presenting an otherwise operable setting.

4. To differentiate the rapidly growing fibro-adenoma from circumscribed mammary carcinoma.

5. To distinguish between recurrent mammary carcinoma and traumatic fat necrosis.

6. To differentiate the thick-walled, deep-seated abscess or cyst from carcinoma. To distinguish diffuse suppurative mastitis from carcinoma accompanied by marked inflammation.

7. To diagnose the deep-seated carcinomatous nodule in fat breasts where palpation is difficult.

Whereas these suggestions offer many problems often settled by the use of an aspirating needle, the procedure is so simple and useful that at the present time almost every breast lesion that presents itself in the breast clinic is aspirated if the least possibility of mammary carcinoma is considered. It requires some degree of skill to obtain cells from a deep-seated tumor, or especially from the sclerosing fibrocarcinomas. If the clinician suspects carcinoma and receives a negative report from his first attempt at aspiration, the report should not deter him from repeating the procedure.

The recognition of breast carcinoma in the smear is relatively simple. Carcinoma cells are large, rather loose and hyperchromatic. They are irregular but tend to appear in loose sheets. Alveolar arrangements are rarely seen. When the tumor cells are small, as they are in the more malignant forms of mammary carcinoma, the chief points in their recognition are their looseness and their irregularity. The cells of a fibro-adenoma may be as large as those of mammary carcinoma; those of proliferated ducts may be as large as the smaller cell types of mammary carcinoma, yet both maintain a coherence (Fig. 4) not found in carcinoma cells (Fig. 5). The spindle cell stroma of some fibro-adenomas permits

their ready detection. Search for mitotic figures has not proved of practical assistance in furthering diagnosis. Other features of the material far outweigh in importance the mitotic figure.

There are borderline pictures that one had best refrain from interpreting. The markedly atypical proliferating and desquamating cells of pronounced Schimmelbusch-Reclus disease may duplicate in smears all the features of mammary carcinoma. The author has once confused these two diseases. The cells of the ducts in ordinary male gynecomastia may offer difficulties. In several cases aspiration of male breasts yielded cells much larger than one would have anticipated from the structure of the normal male breast, yet the cells were uniform in size and definitely coherent. It might be suspected that the lactating breast would be a source of confusion. So far we have had little experience in this field but on two occasions the presence of fat-filled cells and sheets exhibiting an alveolar structure with a double epithelial layer rendered a diagnosis differential from carcinoma possible. It must not be inferred that diagnosis is always simple and that no errors have been made. Until the pathologist has familiarized himself with the various pitfalls errors are certain to occur. They can be corrected only by the knowledge gained from experience. So far, however, the advantages of the method have far outweighed in importance the exceptional error.

There have been 41 aspirations of suspected lung tumors. Aspiration has been used to distinguish between primary carcinoma of the lung and various septic processes, when the lesions are so situated that they are not accessible to the bronchoscope. The recognition of primary bronchogenic carcinoma is simple, but no attempt has been made to distinguish different types, such as epidermoid versus primary adenocarcinoma of mucous glands. The differentiation of primary from metastatic lung tumors has not as yet been required, since radiographs are undoubtedly more decisive in this regard than a smear. In one instance a needle aspiration enabled us to distinguish a recurrent tumor of the pleura, secondary to a peculiar teratoid mixed tumor of the breast, from aneurysm. In another it was possible to detect the daughter cysts of echinococcus in fluid from a dense rounded "tumor" in a patient on whom serological tests for echinococcus had been negative. Save in the case of the last patient, who had a considerable immediate reaction following the evacuation of a rather large amount of fluid, no untoward results have followed

puncturing the lung. In several cases repeated punctures have been required for diagnosis.

The interpretation of smears of pleural or peritoneal exudates contains numerous elements of danger. It is our feeling that such smears are highly unreliable. The difficulty comes in distinguishing tumor cells from mononuclear phagocytic cells and from the sloughing mesothelial lining of the cavity. Cells floating free in fluid become very large and atypical. Multinucleate elements arise. Mitoses occur. Even alveolar structures may be encountered. Unless all these features are taken into account it is unsafe to hazard an opinion that the cells of an exudate are neoplastic. We have hesitated to make that diagnosis even when there was good reason to believe that tumor cells were widely implanted over a serous surface. Occasionally the relation of the cells to a bit of included fibrous stroma may be the decisive point in diagnosis, but such relations are not apt to be found when several liters of fluid are submitted for examination. Sectioned clots from centrifuged exudates are not any more satisfactory than simple smears from the centrifuged fluids.

Aspirations from the thyroid are difficult to interpret. We have examined 45 such specimens. It has been possible to detect the more atypical tumors, such as the giant and spindle cell carcinomas, but the differentiation of papillary or alveolar carcinomas of the thyroid from the more active and hyperplastic forms of Graves' disease, or even the atypical regenerative processes surrounding the colloid adenoma, has not been consistently possible. Struma lymphomatosa is readily confused with the small cell carcinoma of the thyroid, even in sections. Such being the case, it is unlikely that the two conditions can be diagnosed from smears.

Few attempts have been made to secure material from tumors of the abdominal viscera. The liver has been aspirated several times with success, so far as determining the presence of tumor is concerned, but not its actual type. The stomach has been aspirated for verification of tumor, usually at the time of surgical exploration. So far satisfactory material has been difficult to obtain. The kidney region has been needled several times but in each instance to rule out the presence of recurrences in or around nephrectomy scars. On one occasion only has it been possible to determine the type of tumor from the cell morphology.

Aspiration is very useful in distinguishing prostatic carcinoma

from benign lesions of the prostate.³ Much the same problems are presented as in the case of breast tumors. The cells obtained are usually diffusely arranged and distinct alveolar characteristics are lacking. They are larger, disconnected and more atypical than the cells of benign hyperplasia. If tissue is obtained from the epithelium of the major prostatic ducts near their point of entrance into the urethra the appearance is suggestive of cells obtained from fibroadenoma of the breast. It may even suggest epidermoid carcinoma. We have learned to disregard these appearances in prostatic aspirations. To date there have been 104 aspirations of prostates.

Suspected embryonal carcinomas of the testes have been aspirated but 3 times. With the Asheim-Zondek test so uniformly positive in testicular tumors one has a reliable source of confirmation of clinical diagnosis without subjecting these widely disseminating lesions to even the possible danger of the trauma of aspiration.

Aspiration of bone lesions has met with considerable success.⁴ The principal indications for aspiration are to make differential diagnoses between the following types of lesions: (1) benign giant cell tumor and central osteogenic sarcoma; (2) endothelial myeloma, osteogenic sarcoma and osteomyelitis; (3) plasma cell myeloma and metastatic carcinoma; (4) osteogenic sarcoma and metastatic carcinoma. Of these problems the last is by far the most difficult, since a very cellular osteogenic sarcoma (Fig. 7) simulates closely metastatic cancer in smears, especially since the formation of bone or osteoid tissue is not apt to be ascertainable among the unattached cells that usually enter the needle. If cartilage is present it is detected more readily. It is obvious that a diagnosis of benign giant cell tumor may not always be correct, since the malignant osteolytic sarcoma may contain many epulis giant cells, yet the difficulties encountered have been fewer than might have been anticipated. The typical benign giant cell tumor yields large numbers of characteristic multinucleated giant cells that are unmistakable (Fig. 6). Furthermore it must be remembered that the central tumor most apt to be confused with giant cell tumor radiographically is usually of the large spindle cell fibrosarcoma variety, a type rather readily distinguished by aspiration.

Endothelial myeloma in smears shows enormous numbers of small, round or polyhedral cells, either diffusely arranged or in sheets. In some respects it may resemble the smear from a hyperplastic lymph

node. On three occasions we have been able by considerable search to detect the small rosettes characteristic of this tumor (Fig. 8). Osteomyelitis, of course, yields large numbers of inflammatory cells. Plasma cell myeloma is readily diagnosed by the appearance of large numbers of characteristic plasma cells with their typical nuclei and opaque cytoplasm. We have had no experience with aspirating the rarer varieties of bone tumors.

Of the soft part tumors the giant cell tumors of tendon origin are readily diagnosed by their content of epulis giant cells. These cells must be distinguished from the Langhans cells of tuberculous tenosynovitis. Neurosarcoma, for the most part, cannot be differentiated from myosarcoma or liposarcoma, and the harder, more fibrous types of neurosarcoma are apt to yield no cells when aspirated.

The diagnosis of non-pigmented melanoma has not been possible, except with the proper history. One should hesitate to draw any conclusion from the simple occurrence of brownish pigment without employing specific stains for iron, since the steel aspirating needle collects traces of rust, which simulates both blood pigment and melanin. With proper precautions pigmented melanoma can be readily diagnosed.

SUMMARY AND CONCLUSIONS

Needle aspiration is in our experience an expeditious, practical method of tumor diagnosis. In approximately 2500 cases we have observed no untoward result following its use. The interpretation of smears of aspirated material often requires competent clinical assistance. Diagnosis by aspiration is as reliable as the combined intelligence of the clinician and pathologist makes it. The pathologist who ventures to interpret the material obtained by aspiration will have to revise or relearn many criteria. The clinician must appreciate how far the pathologist can logically go in interpreting the smear. Both must maintain a sympathetic attitude toward a new procedure. It is safe to state that in our own institution the method has so established its usefulness that it has acquired a permanent place as a means of diagnosis.

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DESCRIPTION OF PLATES

PLATE 128

FIG. 1. Mucin and cartilage cells aspirated from tumor of scalp.

FIG. 2. Small spindle cells embedded in mucin aspirated from a mixed tumor of the parotid.

FIG. 3. Larger, atypical, irregular hyperchromatic cells aspirated from a malignant submaxillary gland tumor. Same magnification used in Figs. 2 and 3.

FIG. 4. Coherent sheets of rather small, regularly arranged cells obtained by aspiration from a fibro-adenoma of the breast.

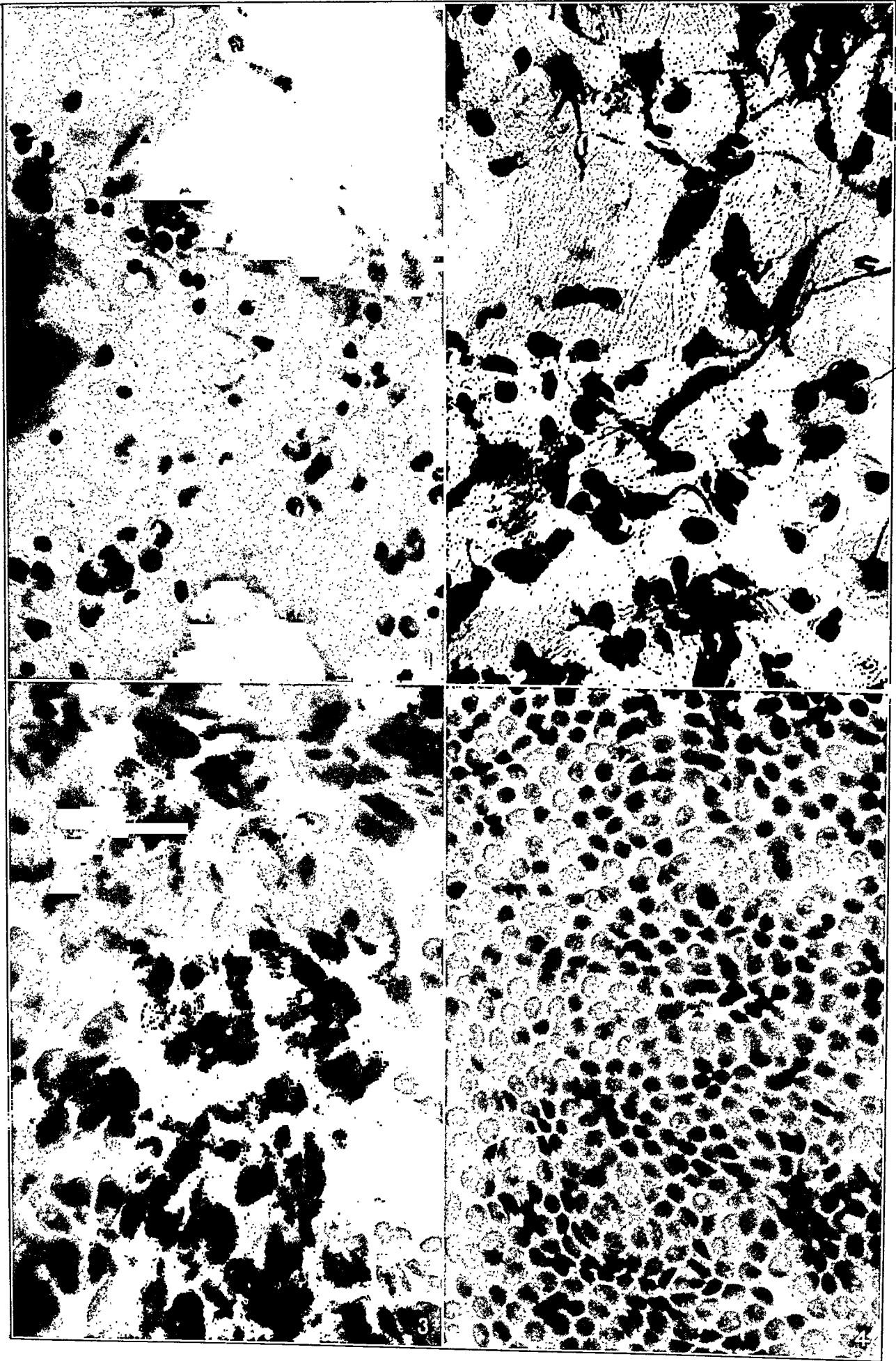
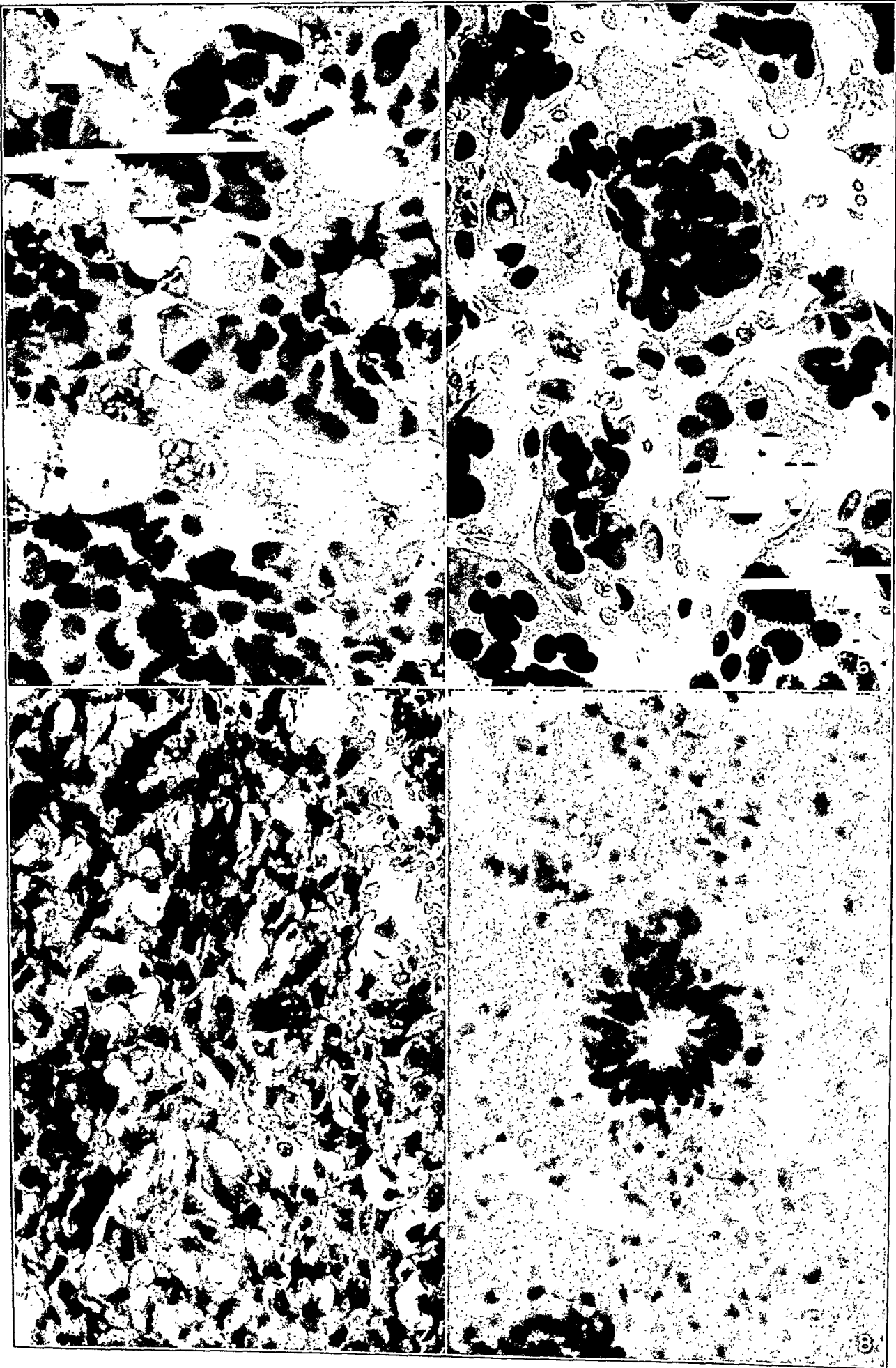


PLATE 129

- FIG. 5. Mammary carcinoma. Same magnification used in Figs. 4 and 5.
- FIG. 6. Benign giant cell tumor of bone. Numerous giant cells of the epulis type are seen.
- FIG. 7. Closely packed, small spindle cells aspirated from an osteogenic sarcoma.
- FIG. 8. Aspirated material from an endothelial myeloma. A typical rosette is seen. Figs. 6, 7, and 8 reproduced at same magnification for comparison.





THE PATHOLOGY OF THE BONE MARROW IN SPRUE ANEMIA*

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INTRODUCTION

The study of bone marrow by means of specimens removed during life has been applied frequently to forms of anemia other than those associated with tropical sprue. Ghedini,¹ Morris and Falconer,² and Zadek^{3, 4} all studied the bone marrow of living patients with pernicious anemia. It remained for Peabody^{5, 6} to apply more modern knowledge to the study of the finer structure of bone marrow. In his final paper he showed most clearly that in pernicious anemia the marrow was hyperplastic during relapse and tended to return to normal during remission. Although the existence of a relation between the macrocytic anemia of sprue and of pernicious anemia has become manifest, existing observations of the bone marrow in sprue with anemia are few in number and confusing. Ashford⁷ briefly reported findings in specimens of tibial marrow studied during life by another worker. Both hyperplastic and aplastic histological pictures were found. No photographs are shown nor are any descriptions of the histological changes given. The author's conclusions as to the marrow pathology seem to be colored largely by existing hypotheses of marrow function based on the response of the peripheral blood to therapy. Mackie and Fairley⁸ described the changes found mainly in the femoral and tibial marrows of a group of autopsied cases of sprue. They noted in certain instances hyperplastic marrow, such as is found in pernicious anemia. In others fatty marrow was found, with rare, circumscribed areas of activity. Still other marrows showed a type of gelatinous degeneration. These various mani-

* These observations were carried out in 1931 by the Commission of the Rockefeller Foundation for the Study of Anemia in Puerto Rico.

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festations were considered to represent various stages, ranging from hyperplastic "active" marrow to atrophic "exhausted" marrow. The fact that the marrow of the long bones is constantly fatty in character under normal conditions and is the last to respond with hyperplasia was not emphasized. Their briefly reported histological studies added little to the descriptions of the gross picture as regards the character of the cellular reaction. However, the red femoral marrow of one patient was stated to have exhibited "production of numerous megaloblasts as well as nucleated red cells."

The most careful and detailed studies of the bone marrow in sprue are those reported by Krjukoff.⁹ This worker studied biopsy material from the ribs of sixteen patients. He was impressed by the constancy with which he found a megaloblastic change of the marrow with the production of "lymphoid erythroblasts" of all sizes, megaloblasts and normoblasts. He does not mention any cases in which an inactive marrow was found. The uniformity of the pathological alterations found is in striking contrast to the observations of other investigators and conforms much better to what is now known to be the effect of adequate specific therapy.

The divergent results of these studies of sprue have, at least in part, been due to the fact that specimens from comparable areas of the marrow were not studied. Piney¹⁰ has shown that only the ribs, flat bones, and vertebral column normally contain active cellular marrow in adult life. Peabody⁵ has emphasized the fact that the marrow of a long bone, such as the tibia, is not necessarily homogeneous in structure and that the pathological process there does not necessarily correspond in extent and degree to that in the marrow of other bones. On the other hand, his own studies of pernicious anemia were carried out on specimens of tibial marrow because he felt that it was simpler in structure and hence presented alterations easier to interpret than the more complicated marrow of flat bones or sternum. After the clarification of the underlying cellular changes of the marrow in pernicious anemia as a result of Peabody's work, it appeared logical to us to examine a site of normally active marrow in sprue. It was felt that in this way early alterations might be observed more readily and conclusions drawn with more certainty as to the presence of hyperplasia or aplasia of the functional elements. For these reasons this communication is based largely upon examination of specimens of sternal bone marrow removed at operation.

METHODS

Twenty-two patients with sprue anemia of a macrocytic type were studied. In certain instances specimens were removed both before and after remissions, in others during the height of the reticulocyte response to liver therapy. The eventual death of three patients made it possible to compare the postmortem pathological picture with that seen during life. In still another group abnormalities of the clinical course were correlated with the histopathological alterations of the marrow.

The procedure of removing the specimens of sternal marrow was carried out in the operating room under complete surgical precautions for asepsis. A longitudinal midline incision about 4 cm. long was made from the third to the fifth costal cartilages, and was extended down to the periosteum. Two incisions were made in the periosteum, each about 1 cm. long and at right angles to each other. The periosteum was carefully retracted to expose an area of bone about 1 cm. in diameter. With a small crown trephine the outer table of bone was removed as a button about 0.4 cm. in diameter. With a bone curette enough marrow was removed to ensure the inclusion in microscopic sections of actual marrow tissue. The bone button was replaced and hemorrhage from the bone stopped with bone wax. The periosteum was brought together with No. 0 plain catgut. One or two subcutaneous stitches of the same suture material were used to approximate the subcutaneous tissue and the skin was closed with interrupted silk sutures. A dry dressing applied with considerable pressure from heavy adhesive straps was kept in place for twenty-four hours.

The tissue was fixed immediately in Zenker's fluid. After the usual steps of dehydration the tissue was embedded in paraffin and cut at 6 microns without decalcification. Mallory's phosphotungstic acid hematoxylin, eosin-methylene blue and the Giemsa stain were employed. Certain blocks were cut serially.

RESULTS

The results of the microscopic examination of the bone marrow are summarized in the table and will be discussed below. In addition a few illustrative cases are briefly cited, and the microscopic picture described in detail.

TABLE I

Table Showing Blood Picture and Bone Marrow Histology in Macrocytic Anemia of Sprue

Case No.	Blood picture						Bone marrow					Comment		
	Red blood cells in millions	Hemoglobin per cent	White blood cells in thousands	Reticulocytes per cent	Mean corpuscular volume in cu. μ	Icterus index units	Color index	Biopsy or postmortem	Bone source	General cellularity	Megalo-blasts and erythro-blasts		Normo-blasts	Fat
Untreated Cases														
1	2.15	54	8.1	1.8	130	2	1.25	Biopsy	Sternum	++	++	++	++	Very early though definite megaloblastic preponderance (Fig. 1).
2	1.76	49	6.2	1.4	126	5	1.39	"	"	+++	+++	+	+	Moderate megaloblastic proliferation and preponderance (Fig. 2).
3	1.41	40	3.3	3.2	126	4	1.42	"	"	++++	++++	-	-	Intense megaloblastic proliferation and preponderance (Fig. 3).
4	1.42	48	2.2	6.6	140	40	1.69	"	"	++++	++++	-	-	"
5	0.98	28	2.2	0.8	143	25	1.43	"	"	++++	++++	-	-	"
6	2.51	55	3.3	4.8	101	2	1.10	"	"	++++	++++	-	-	"
7	1.96	46	9.8	1.4	114	2	1.17	"	"	++++	++++	-	-	"
8	1.92	42	4.8	2.0	102	5	1.10	"	"	++++	++++	-	-	"
9	2.67	66	7.4	2.2	119	3	1.24	"	"	++++	++++	-	-	"
10	1.15	34	5.1	3.8	131	30	1.48	"	"	++++	++++	-	-	"
11	2.52	63	8.1	3.2	128	3	1.25	"	"	++++	++++	-	-	"
12	3.12	73	7.5	1.6	107	2	1.14	"	"	++++	++++	-	-	"
13	1.25	29	1.8	1.0	104	18	1.16	"	"	++++	++++	-	-	"
14	1.68	55	5.0	4.6	134	5	1.63	"	"	++++	++++	-	-	"
15	2.40	54	1.9	0.4	113	40	1.12	"	"	++++	++++	-	-	"
16	1.46	40	3.0	0.1	121	5	1.37	"	"	++++	++++	+	+	Moderate megaloblastic proliferation and preponderance.

17	1.05	29	2.9	8.8	157	12	1.38	Postmortem	Sternum and femur	++++	++++	-	-	Phagocytosis of red cells present (Fig. 6).
18	2.86	68	7.6	1.6	119	3	1.19	"	"	++++	++++	-	-	Sternal marrow red, femoral partly red. Lung abscess. Microscopically very loose structure.
19	0.97	19	7.3	4.5	103	30	0.98	"	"	++++	++++	-	-	Brick red marrow sternum and upper third of femur. Transfusion. Microscopic structure confused. Phagocytized erythrocytes.

Treated Cases

20	0.94	19	3.6	4.4	115	10	1.00	1st biopsy	Sternum	++++	++++	+	-	Before remission. Many megaloblasts, few normoblasts (Fig. 4), almost no fat cells.
20	2.97	60	3.3	4.2	100	2	1.01	2nd biopsy	"	++	+	+++	++	20 days after intramuscular liver extract. Predominant normoblasts (Fig. 5).
21	0.83	26	3.0	14.0	162	3	1.57	Biopsy	"	++	++	+	+	9th day oral liver extract. During reticulocyte rise. Megaloblasts separating into clumps. Normoblasts appearing.
22	1.40	29	2.3	21.6	114	6	1.04	1st biopsy	"	+++	++	++	+	9 days after liver extract intramuscularly. During reticulocyte rise. Decrease in megaloblasts. Increased normoblasts.
22	2.69	55	2.8	2.0	99	3	1.02	2nd biopsy	"	+	+	+++	+++	30 days after liver extract. Late remission. Marrow picture approaches normal, except for increased normoblasts.

Cellular Composition of the Sternal Bone Marrow in Untreated Cases of Sprue

CASE 1. Clinical History: The patient was a Puerto Rican housewife 47 years of age. There was a history of fairly typical sprue for one year, with loss of weight, gastro-intestinal disturbance, glossitis and anemia. Before the biopsy the patient had been treated for sixteen days with autolyzed yeast, to which she showed a slight reticulocyte response and slight clinical improvement. By the time of the biopsy the lingual and gastro-intestinal symptoms were somewhat improved without significant changes in blood values.

On the day of the biopsy, Aug. 26, 1931, examination of the blood showed: Red blood cells 2,150,000 per cmm.; hemoglobin 54 per cent (Sahli); color index 1.25; mean corpuscular volume 120 cu. μ ; leukocytes 8100 per cmm.; reticulocytes 1.8 per cent; icterus index 2. The blood smear was consistent in appearance with the macrocytic anemia of sprue.

Bone Marrow Biopsy: The tissue appeared only moderately cellular and contained quite a large number of bone spicules. It was red-brown in color. The microscopic appearance is illustrated in Figure 1. A considerable amount of fat is present. The number of cells is distinctly greater than that observed in normal sternal marrow. Groups of from six to eight megaloblasts are seen with round nuclei containing rather heavy masses of chromatin and occasionally in mitosis. Certain cells of this general type are larger, with a considerable amount of rather pale, basophilic, cytoplasm. The latter is the type of cell that is present in such large numbers in the bone marrow in pernicious anemia in relapse. Smaller cells, diffusely scattered, with round nuclei containing dense masses of chromatin are numerous. These have a heavily basophilic cytoplasm that varies greatly in amount, though it is never so great as in the megaloblast. These cells are considered to be erythroblasts of varying degrees of maturity. A considerable number of normoblasts is present, many more than are seen in the marrow of pernicious anemia in relapse or in marrows from patients with sprue of a more severe degree. Megakaryocytes are present in about normal numbers, as are cells of the granulocytic series. Many sinusoids are closed.

Comment: Subsequent heavy dosage with liver extract administered intramuscularly failed to effect a reticulocyte rise or improvement in blood values. When ferric ammonium citrate was administered, however, a distinct reticulocyte rise appeared and subsequently a return to normal blood levels resulted. In this marrow the frequent occurrence of normoblasts suggests a deficiency of iron

as well as of liver extract, which is confirmed by the fact that both liver extract and iron were required to bring about improvement. In contrast to the findings in the bone marrow of Case 2, the histological changes are slight. It is conceivable that this is the earliest change, since the illness of the patient was short, and the blood values were at a relatively high level.

CASE 2. Clinical History: The patient was a male journalist 60 years of age, who had suffered from gastro-intestinal disturbance, glossitis, and weakness for ten years. For the ten days preceding the biopsy the patient was treated with 6 gm. of ferric ammonium citrate daily without effect.

On the day of the biopsy, Sept. 8, 1931, the blood examination showed: Red blood cells 1,760,000 per cmm.; hemoglobin 49 per cent (Sahli); color index 1.39; mean corpuscular volume 126 cu. μ ; white cells 6200; reticulocytes 1.4 per cent; icterus index 5. The blood smear was typical of the macrocytic anemia of sprue.

Bone Marrow Biopsy: The marrow did not appear remarkable at operation. Some increase in cellular over bony tissue was present and the color was distinctly reddish. The microscopic appearance is illustrated in Figure 2. A moderate amount of fat is present, up to thirty cells per high power field. Between the fat cells are masses of cellular tissue. The vast majority of the cells present are megakaryoblasts, some arranged in groups and others scattered diffusely throughout. They are larger, in general, than are those seen in Case 1 and have considerably more cytoplasm. Mitoses are frequent. Many erythroblasts of varying degrees of maturity are seen. The relative number of normoblasts is strikingly decreased. A few cells of the granulocytic series are present. The marrow is similar to that seen in pernicious anemia in relapse, except for the lack of complete replacement of fat by cellular tissue. The sinusoids are open and contain many adult red cells. The uniformity of the cellular picture is most striking. Megakaryocytes are sharply reduced in number.

Comment: The administration of ferric ammonium citrate effected no improvement. Liver extract given orally brought about a slight reticulocyte rise and by subsequent parenteral administration restoration of normal blood values was slowly attained. This result could perhaps have been prognosticated from the changes present in the marrow. That the response was a slow one might be associated with the fact that although the cell type was uniform, complete replacement of fat had not taken place. This marrow is intermediate in

type between that of Case 1, where megaloblastic activity was mild and early, and much fat present, and that of Case 3, in which a picture quite similar in all respects to that of pernicious anemia was seen.

CASE 3. Clinical History: The patient was a Puerto Rican housewife, 60 years of age, who had suffered from malnutrition for one year, and during the past three months from glossitis, diarrhea, and edema of both feet. There had been considerable loss of weight.

On the day of the biopsy, Aug. 26, 1931, the blood examination was as follows: Red blood cells 1,410,000 per cmm.; hemoglobin 40 per cent (Sahli); color index 1.4; mean corpuscular volume 125 cu. μ ; white blood cells 3300 per cmm.; reticulocytes 3.2 per cent; icterus index 4. The blood smear was typical of the macrocytic anemia of sprue.

Bone Marrow Biopsy: Grossly the marrow appeared dark red and hyperplastic. Definite replacement of bone spicules had taken place. Little fat was seen. As shown in Figure 3, microscopic examination bears out this impression. Almost no fat is present, averaging fewer than one fat cell per high power field. The tissue is rather vascular and extremely cellular. Masses of large megaloblasts are present. These cells are somewhat irregular in size and outline. The nuclei are rounded or oval and contain rather little chromatin, that present being clumped in irregular masses. The cytoplasm is slightly basophilic and irregular in outline. Smaller clumps of cells of somewhat different character are seen. These cells have round nuclei with heavy masses of chromatin. The cytoplasm varies in amount and is deeply basophilic. These cells are considered to be erythroblasts. Very few normoblasts are seen. A moderate number of megakaryocytes is present, as is some myelopoiesis with all stages in the development of the granulocytic cell series.

Comment: The picture is one of diffuse megaloblastic hyperplasia, similar in nature to that seen in pernicious anemia in relapse. As might be expected with the type of bone marrow change described, a prompt reticulocyte response followed the intramuscular injection of liver extract. This response was followed by eventual restoration of normal blood values.

In the three cases just described different degrees of the same fundamental histological change have been observed. The presence of such definite variations in the intensity of the process, even in the sternal marrow, is considered to be a partial explanation of the failure of many investigators to find active marrow in the long bones of

certain autopsied cases of sprue anemia. Presumably, in such instances, the pathological change has failed to extend to the long bones, though strikingly present in the sites of most active blood formation where the earliest changes would be expected to appear. In no case examined, and many, as may be seen from the table, had anemia of an extreme degree, was the sternal bone marrow atrophic. This is in agreement with the observations of Krjukoff and quite opposed to the statements of those who have confined their studies to the marrow of the long bones.

The Effect of Therapy on the Cellular Composition of the Sternal Bone Marrow in Sprue

Hitherto the marrow in cases of sprue anemia has not been studied before and after treatment capable of producing reticulocyte rises and restoration of normal blood values. Two cases of the present series were subjected to sternal punctures at different stages of the disease and the histological pictures compared. The changes were similar in both cases.

CASE 20. *Clinical History:* The patient was a male Puerto Rican, 35 years of age, who entered the hospital complaining of weakness of six months duration. For four months he had suffered from watery diarrhea and glossitis. The day before the first biopsy he received a transfusion of 200 cc. of whole blood and an injection of an effective extract derived from 100 gm. of liver.

On the day of the first biopsy, Aug. 6, 1931, a blood examination showed: Red blood cells 940,000 per cmm.; hemoglobin 19 per cent (Sahli); color index 1.0; mean corpuscular volume 115 cu. μ ; white blood cells 3600 per cmm.; reticulocytes 4.4 per cent; icterus index 10.

First Bone Marrow Biopsy: In the gross the tissue was soft, deep red, and had definitely replaced a large part of the bony trabeculae. Histologically, as shown in Figure 4, all resemblance to normal marrow structure is lost. No fat is present. The tissue is an almost solid mass of large, pale cells, with nuclei containing scattered masses of chromatin. Their outline is irregular, the cytoplasm moderately basophilic, and the cells are in close apposition. These cells are considered to be megaloblasts. Scattered diffusely throughout in large numbers are somewhat smaller cells with round nuclei containing dense and heavy chromatin. The cytoplasm of these cells is small in amount and is somewhat more deeply basophilic. Many mitoses are present. Nucleated red cells are almost entirely absent. Myelo-

poiesis is slight. Only a limited number of megakaryocytes are present. The vascular channels are obscured by the enormous cellular overgrowth. The whole picture is quite consistent with that seen in severe pernicious anemia.

The effect of liver extract on this patient was entirely similar to that occurring in Addisonian pernicious anemia. In response to the single injection of liver extract derived from 100 gm. of liver the reticulocytes reached a peak of 38 per cent on the fifth day and the blood values rose rapidly, as similar therapy at ten day intervals was maintained.

Twenty days after the first biopsy and after remission had occurred, though complete restoration of the blood levels to normal had not taken place, a second biopsy was performed. On the day of the second biopsy, Aug. 26, 1931, the blood examination showed: Red blood cells 2,970,000 per cmm.; hemoglobin 60 per cent (Sahli); color index 1.02; mean corpuscular volume 100 cu. μ ; white blood cells 3300 per cmm.; reticulocytes 4.2 per cent; icterus index 2.

Second Bone Marrow Biopsy: Histologically, the picture is quite different from that of the first biopsy, as shown by a comparison of Figure 4 with Figure 5. Fat cells are present up to ten to twelve per high power field. A few large pale megaloblasts with irregular nuclei are present in groups of three or four. The solid background of these cells seen in the first specimen is completely replaced by sheets of normoblasts that greatly outnumber any other cell type present. Vascular channels are open, myelopoiesis is proceeding, and a fair number of giant cells may be seen. Clearly, the administration of effective therapy caused a maturation of megaloblasts to normoblasts with the production of a bone marrow morphology more nearly approaching the normal and so resembling the transition observed during the treatment of pernicious anemia.

Phagocytosis of Erythrocytes by Bone Marrow Cells in Sprue

The presence of erythrocytes in the cytoplasm of large cells of the bone marrow in pernicious anemia has been described frequently in specimens of marrow removed at autopsy. At a time when current views associated pernicious anemia with increased blood destruction, based upon the observations of the increased serum bilirubin content and an output of bile pigment greater than normal in the

excreta, Peabody considered that phagocytosis might be of significance in the production of the anemia. In an early paper Peabody and Broun ¹¹ gave a detailed description of the phagocytic picture in pernicious anemia, as seen in tissue fixed postmortem, and compared it with postmortem material from other types of disease. Although phagocytosis occurred, especially in infectious diseases, it was apparently greater in cases of pernicious anemia where death occurred in an acute stage. Later observations of biopsy material, however, failed to reveal erythrocytes in the cytoplasm of large marrow cells and led to the conclusion that the cytological appearances, which were supposed to indicate the phagocytosis of red blood cells during life, were really postmortem, or at least terminal phenomena.

Study of postmortem specimens of bone marrow from two cases of sprue anemia in Puerto Rico revealed the same phenomenon, so frequently observed in marrows of patients dying of pernicious anemia. In both instances erythrocytes are seen in the cytoplasm of large bone marrow cells, as shown in Figure 6. In both of these specimens the architecture of the marrow is seriously distorted, as compared with tissue removed at biopsy. In the latter, closely packed masses of cells, uniform in size and shape and easily identifiable, with well marked vascular channels and stroma structure are seen. In the postmortem specimens the structure is extremely loose and almost unrecognizable. There is an enormous variation in size and shape of the cells. Some are very large and have an irregular, slightly basophilic cytoplasm, which contains large numbers of erythrocytes. Since this was not observed in any of the specimens removed at biopsy the conclusion is unavoidable that it is a postmortem change. In neither case was the body kept more than eight hours before the tissue was fixed, although lack of facilities for refrigeration and the heat of the climate may have accelerated a postmortem alteration. Peabody states that the cells that ingest erythrocytes in the bone marrow of pernicious anemia are clasmatoocytes. He quotes the observation of Rich ¹² that clasmatoocytes may ingest red cells when grown *in vitro*. From the observations here presented no conclusion as to the nature of the phagocytic cell can be drawn. Only further evidence, based on supravital and tissue culture study of material removed at autopsy, can be expected to solve the problem.

DISCUSSION

The material presented indicates that, as concerns the fundamental histopathological picture of the bone marrow during exacerbation, during remission and at postmortem, the changes accompanying the macrocytic anemia of tropical sprue are similar to those found in pernicious anemia, as described by others.

Peabody confirmed the previous observation of Zadek that during remission the bone marrow became less cellular; and for the first time accurately described the microscopic appearances both during remission and in relapse. He considered the hyperplasia occurring in relapse to be an extensive proliferation of primitive marrow cells, chiefly megaloblasts, associated with a relative decrease in the other elements, including the fat cells. Remission was shown to be characterized by a picture more nearly normal, with few megaloblasts, a relative increase of normoblasts and mature red blood cells, and the reappearance of fat. The anemia of relapse was explained by the functional ineffectiveness of the marrow resulting from the failure of the megaloblasts to form mature erythrocytes. In the sternal marrow of nineteen patients with untreated sprue the same fundamental pathological change was found, an increase in number and size of the megaloblasts, a decrease in the amount of fat present and in the number of megakaryocytes and cells of the granulocyte series. The number of normoblasts was strikingly small in comparison to the number of megaloblasts. The differences in the bone marrow changes reported by previous observers can thus apparently be explained by the fact that only the marrow of the long bones was studied. Beyond question the extent of marrow involvement is greater in pernicious anemia than in certain cases of sprue, but the changes occurring in the normally active marrow of the adult, obtained here by sternal biopsy, present a uniform picture.

Furthermore, the effect of specific therapy in sprue anemia is followed by the same maturation of megaloblasts to normoblasts and restoration of normal morphology of the marrow, as occurs in pernicious anemia. This fact parallels the similarity of the blood changes in the two conditions. Although, as a rule, the effect of liver extract on the lingual and gastro-intestinal symptoms is as striking as in pernicious anemia, the hematopoietic response is frequently not so marked for a given blood level in sprue as in per-

nicious anemia. This is probably to be explained, at least partly, on the basis of the fact that in pernicious anemia the megaloblastic hyperplasia involves a greater amount of the normally inactive bone marrow. It is also clear that in sprue a combination of therapy with iron, as well as with liver extract, is necessary in many cases, a fact that correlates with the findings in the bone marrow of certain patients, as illustrated by Case 1. Despite this fact, the basic similarity of the blood pictures and of the changes in the active portions of the bone marrow in relapse and in response to similar therapy would seem to be strong evidence for a similar etiological mechanism. Observations have been made by Castle and Rhoads¹³ that indicate the macrocytic anemia of sprue is mainly the result of a deficiency similar to that existing in pernicious anemia, although brought about in a somewhat different manner.

CONCLUSIONS

1. Observations on the bone marrow of sprue anemia made on tissue obtained at biopsy in different stages of the disease show that the changes are similar to those of pernicious anemia.
2. During relapse the essential change is a proliferation of megaloblasts and suppression of maturation to the normoblast stage.
3. During remission the marrow tends to return to normal with a great increase in the number of normoblasts and mature red cells in the marrow.
4. Phagocytosed erythrocytes were observed in the bone marrows removed at autopsy but not in those removed during life.

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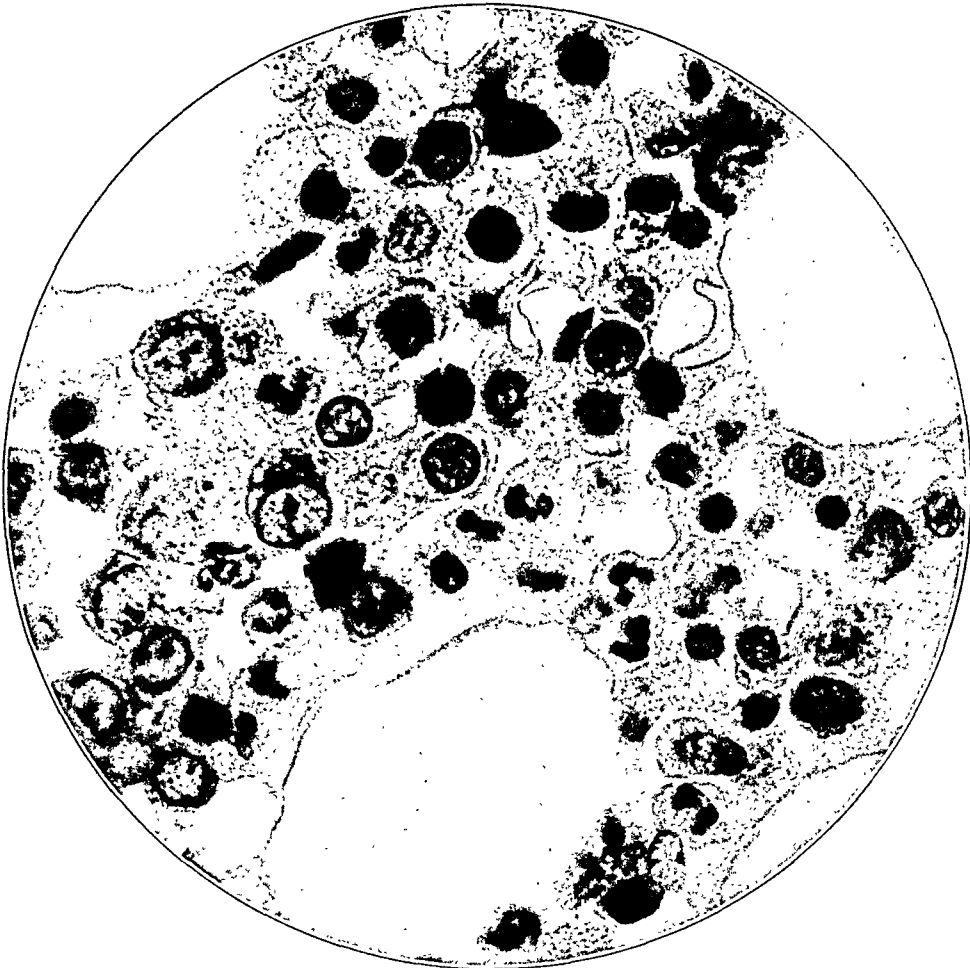
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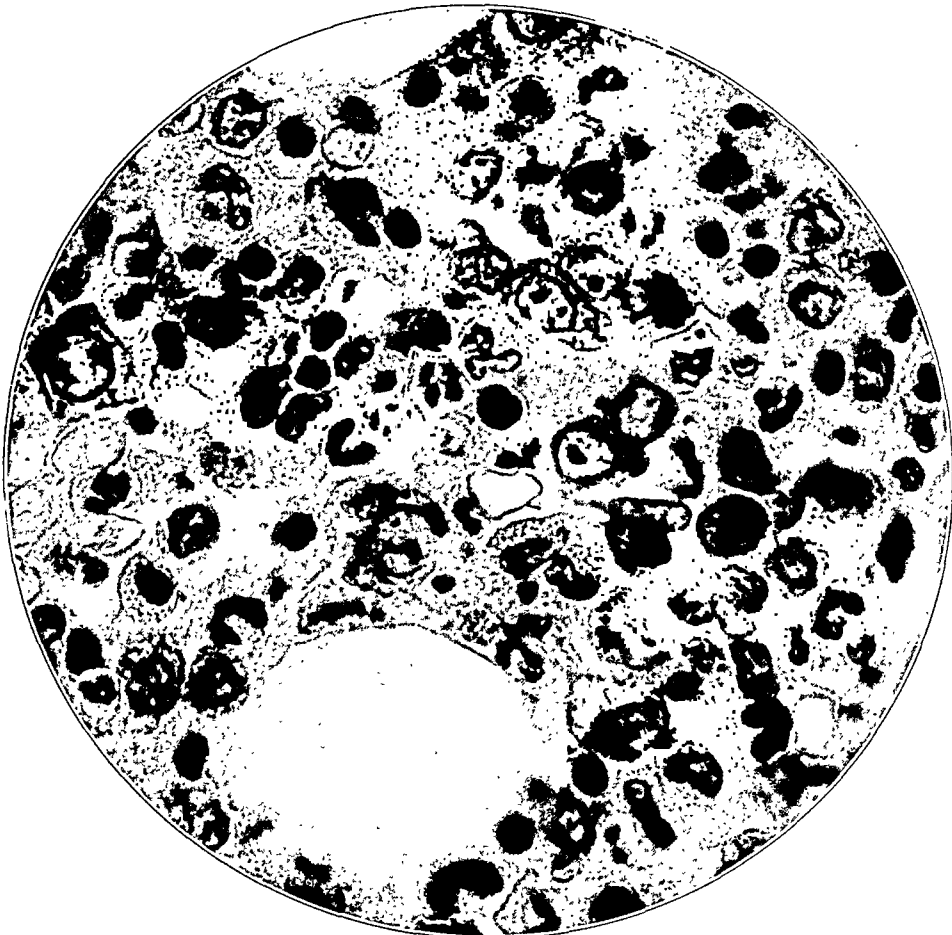
PLATE 130

FIG. 1. Case 1. Sternal marrow removed at biopsy from patient with macrocytic anemia of sprue of moderate degree and duration before remission produced subsequently only after addition of iron to liver extract therapy. Note presence of much fat, numerous normoblasts and moderate numbers of megaloblasts. Giemsa stain. $\times 1500$.

FIG. 2. Case 2. Sternal marrow removed at biopsy from patient with macrocytic anemia of sprue. Note presence of fat and moderate megaloblastic preponderance. Cells of the myeloid series are present in considerable numbers. Giemsa stain. $\times 1000$.



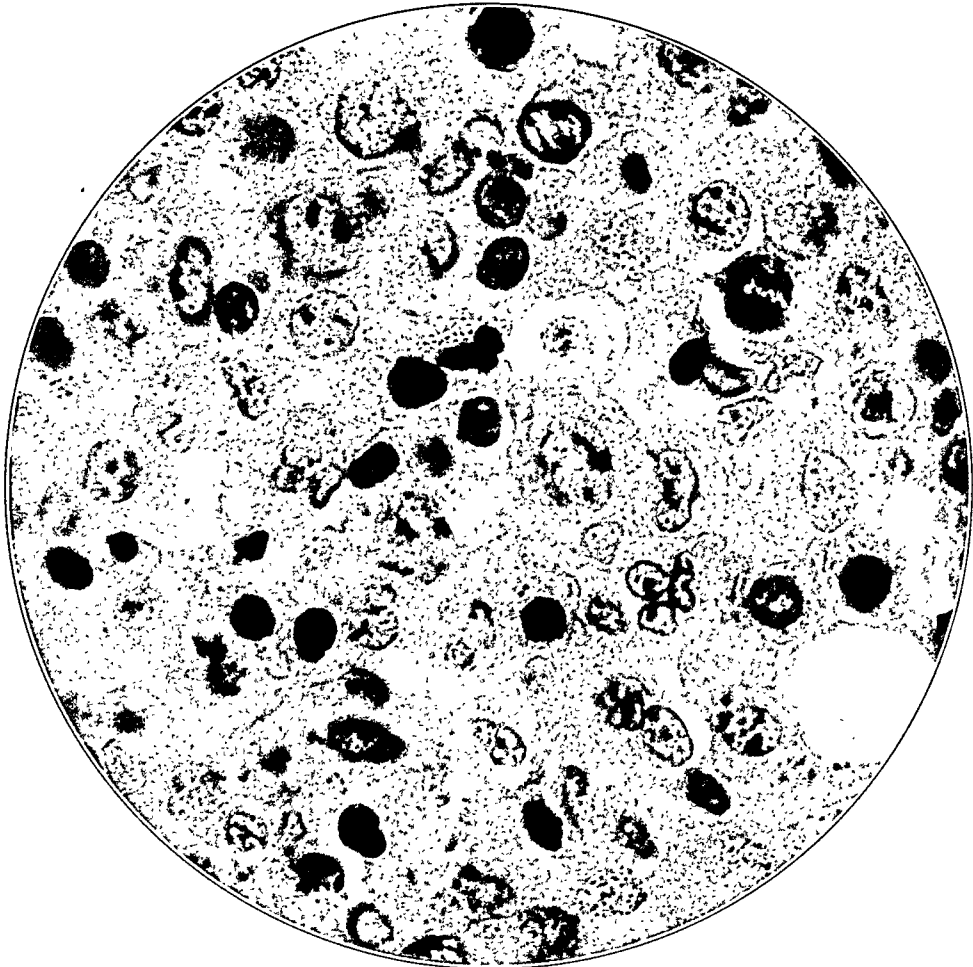
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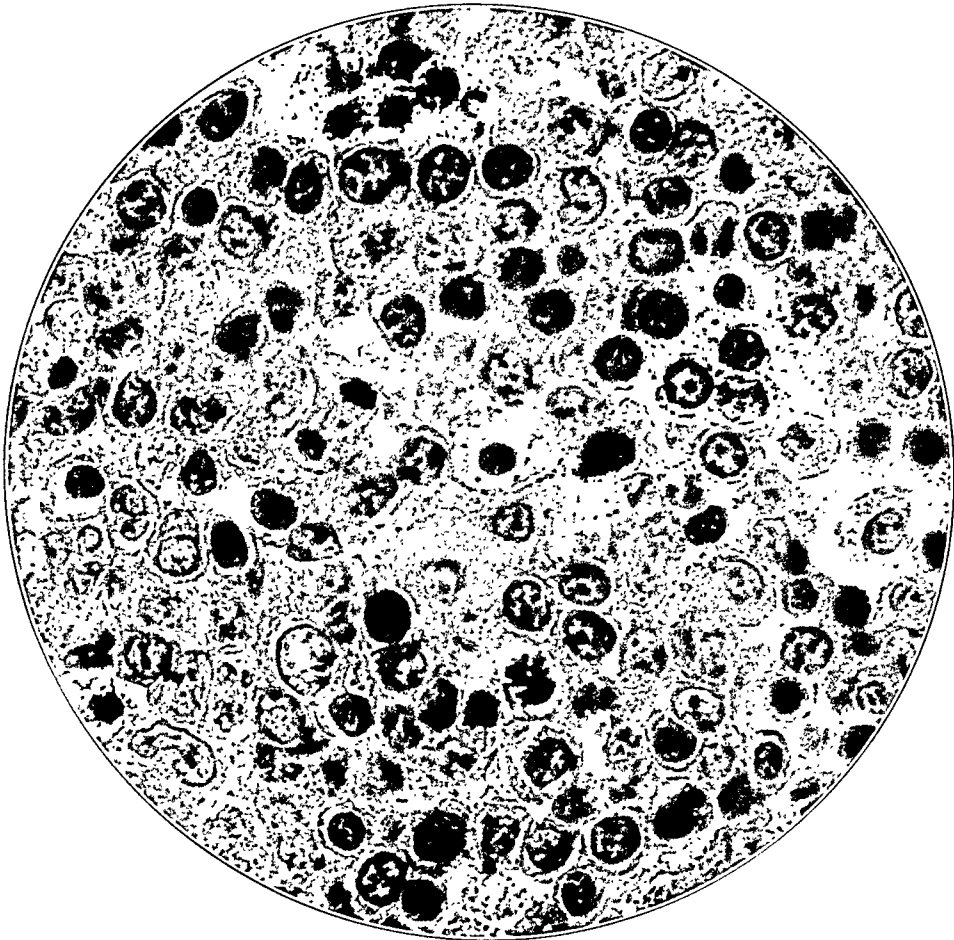
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PLATE 131

- FIG. 3. Case 3. Sternal marrow removed at biopsy from patient with severe macrocytic anemia of sprue. Note almost total absence of fat and intense megaloblastic proliferation with relatively few normoblasts and cells of myeloid series. Giemsa stain. $\times 1500$.
- FIG. 4. Case 20. Sternal marrow removed at biopsy from patient with severe macrocytic anemia of sprue before typical remission produced with liver extract. Note absence of fat with increased cellularity due largely to intense megaloblastic proliferation with occasional mitotic figures. Giemsa stain. $\times 1000$.



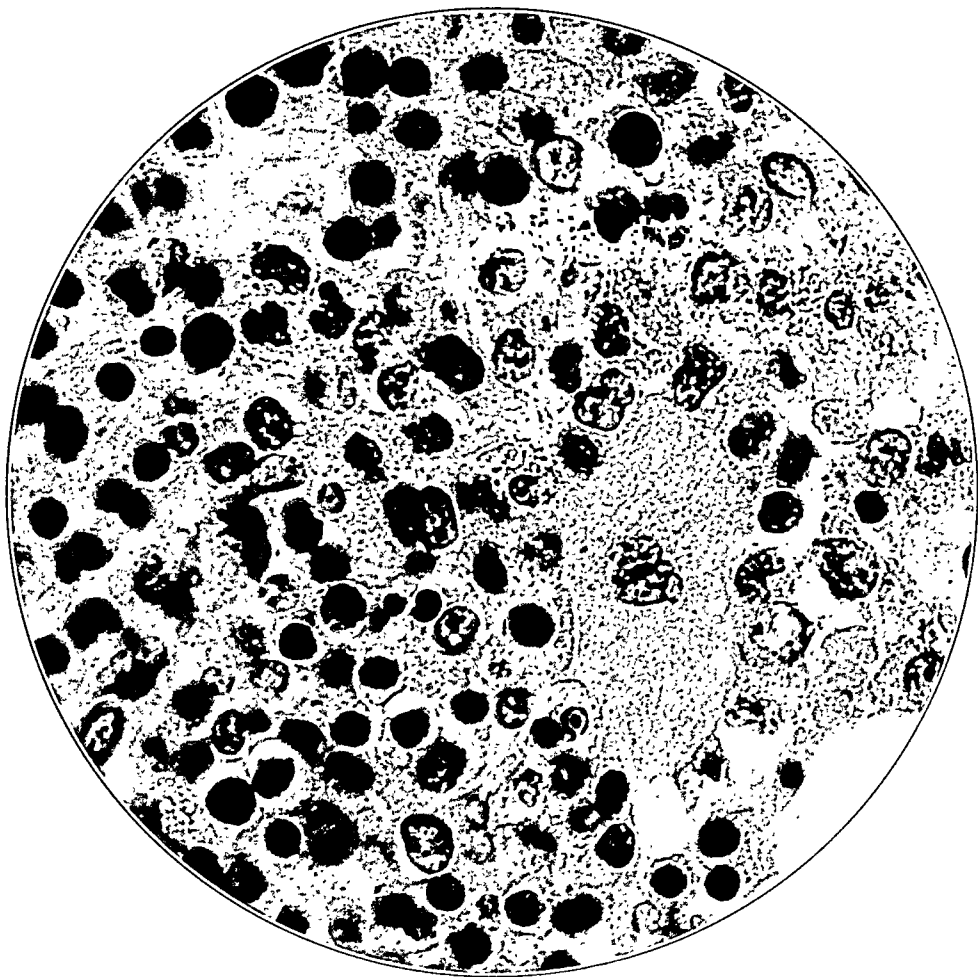
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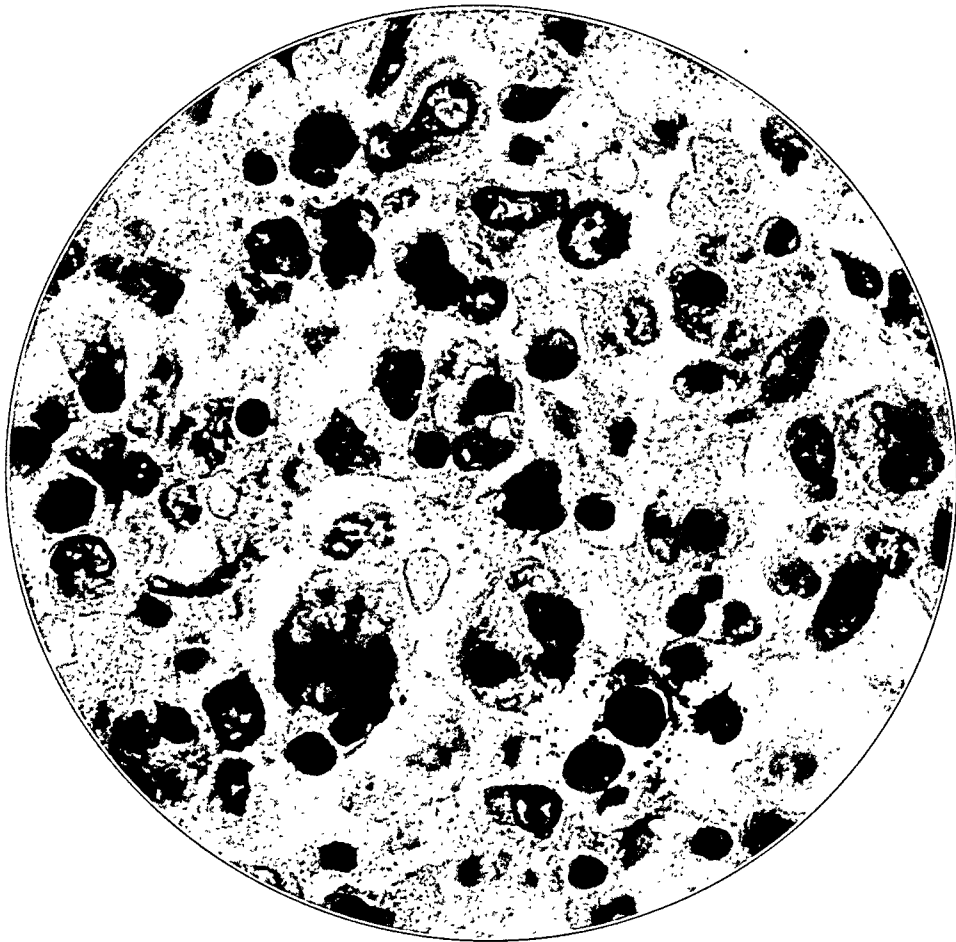
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PLATE 132

- FIG. 5. Case 20. Second sternal marrow removed at biopsy twenty days after injection of active liver extract, which produced a typical reticulocyte crisis. Note the decreased number of megaloblasts and the normoblastic preponderance. Giemsa stain. $\times 1000$.
- FIG. 6. Case 17. Sternal marrow removed at autopsy from patient dying of sprue with severe macrocytic anemia. Note loss of structural details and presence of phagocytosed erythrocytes. Giemsa stain. $\times 1000$.



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MELANOMA STUDIES *

III. A THEORY OF PIGMENTED MOLES. THEIR RELATION TO THE EVOLUTION OF HAIR FOLLICLES

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- II. The reptilian tactile spot
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I. THE PIGMENTED MOLE AS A TACTILE TUMOR

Discussion of the origin of pigmented moles should begin with a mutual understanding of just what a pigmented mole is. All readers will agree, we believe, that the pigmented mole is an abnormal growth in human skin, that it is pigmented, usually elevated, often hairy, and that on microscopic examination groups of nevus cells are found in the corium. We shall make the further claim that the pigmented mole shows distinct traces of having been at one time a tactile organ with a full complement of sensory nerves and tactile terminals. It is at this point that differences of opinion arise. It is at this point therefore that we shall begin to present our evidence.

Nerve Terminals in Moles

Any good neural silver stain applied to a pigmented mole will reveal branching nerve trunks, as pictured in Figure 1. There is

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nothing unusual about a nerve trunk branching in human skin. All normal skin offers these pictures. We would direct attention to the smaller branch which leaves the crotch of the main trunk and passes to the right. This nerve divides and subdivides into fine threads. The threads terminate in tiny bulbs in contact with some of the nevus cells which form the background of the picture. This is exactly the manner in which many nerves terminate among the Merkel-Ranvier tactile cells of the hair follicles and the basal cells of the epidermis. Such a distribution of nerve fibers is never found in normal human corium but it is a common sight in pigmented moles.

Figure 2 shows a more elaborate distribution of nerves in another mole. The background is a solid sheet of nevus cells among which the nerve fibers ramify, the fine threads winding around the nevus cells and terminating as tiny end-bulbs in intimate contact with some of them.

Figure 3 shows another neural distribution which is common in hairy moles. The most prominent feature of the picture is the hair in its follicle. At the left of the follicle a nerve divides. Some of the branches pass upward and to the right to supply the follicle. These nerves terminate in tiny end-bulbs in contact with epithelial cells of the follicle, which consequently are regarded as cells specialized for the reception of tactile impulses. Other branches diverge upward to the left and downward to the right. These branches terminate in end-bulbs on and among the nevus cells. If the epithelia of the hair follicle are accepted as specialized tactile cells by virtue of their innervation it is a fair proposition that the nevus cells also are tactile cells by virtue of an exactly similar innervation. Pictures of such joint innervation of hair follicles and nevus cells might be multiplied indefinitely.

The tactile nature of these neural nerves was demonstrated in another way by Masson (1926, 1931, 1932),^{1, 2, 3} as shown in Figure 4, a Masson-Mallory trichrome stain of a paraffin section of a mole from the occiput. The section is from Professor Stout's collection. Like many moles on or near the scalp, this one is crowded with figures which Masson first described and, we believe, interpreted rightly as caricatures of Meissnerian tactile corpuscles. Masson's demonstration of tactile corpuscles in pigmented moles has been corroborated by Stout (1932),⁴ Ewing (1928, 1930),^{5, 6} Foot (1931),⁷ who

presented convincing preparations at the meeting of the American Association of Pathologists and Bacteriologists in Philadelphia last year, and by Frantz and McFarland (personal communications).

Figure 5 shows another common sight in pigmented moles when stained for nerve fibers. It is what we call the cable effect. Around a few neurites traversing the colorless corium there is an enormously thick sheath of cells. Such pictures are characteristic of von Recklinghausen's neurofibroma of the skin; in fact, it was this formation that led Soldán (1899)⁸ to declare unequivocally that the pigmented mole is a neurofibroma. Using Weigert's myelin stain Soldán demonstrated medullated fibers only; he did not use silver.

Figure 6 is a normal meissnerian corpuscle from the sole of the human foot. It is introduced for two purposes, to show that our staining technique is actually specific for nerve fibers and to demonstrate that, with this technique at least, the nerves are seen to terminate among the well recognized tactile cells of the meissnerian corpuscle exactly as they do among the nevus cells.

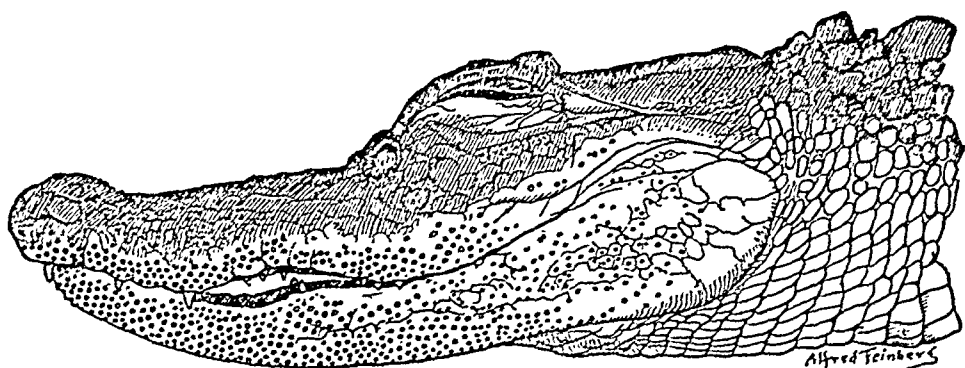
Assuming that we have come to an agreement that pigmented moles contain sensory nerve fibers and cells that appear to be tactile cells because of their neural contacts, together with structures resembling meissnerian tactile corpuscles, let us pass to a consideration of their possible origin. For all tumors or for almost all tumors, there is a prototype, that is, some adult or embryonic tissue of which the tumor is a more or less faithful reproduction. It is a striking feature of pigmented moles that they do not resemble anything known in normal mammalian skin. No normal mammalian corium contains groups of cells, innervated, pigmented and elevated in the manner characteristic of pigmented moles. If, however, we look at the creatures just below the mammals in the phylogenetic scale, the reptiles and the amphibia, we shall find them peppered with pigmented dots which on microscopic examination present the structure of human pigmented moles.

II. THE REPTILIAN TACTILE SPOT

Text-figure 1 represents the head of the common alligator. Along the upper and lower jaws there are many black dots standing out prominently on the smooth, white skin. Each dot is a sensory nerve terminal, generally accepted by zoölogists as a tactile organ. Such tactile organs are common to reptiles and amphibia. They were dis-

covered in the frog by Merkel (1880)⁹ and called by him *Tastfleck*, tactile spots.

Figure 7 is a vertical section through one of these tactile spots stained for nerve fibers. You will note that the tactile spot is elevated and pigmented, and that there is a group of cells in the corium just beneath the epidermis. These are Merkel-Ranvier tactile cells mixed with Schwann cells from the nerve fibers. The ascending nerve breaks into fine terminal threads which wander among the tactile cells just as similar terminal threads wander among the nevus cells. Some of the threads terminate as end-bulbs, some as menisci, in contact with a tactile cell; some continue on into the epidermis. You will recognize the resemblance of this structure to that of the



TEXT-FIG. 1. Alligator Mississippiensis

innervated pigmented mole. There is the same elevation, the local accumulation of pigment, the group of tactile cells in the corium and the fine nerve fibers winding among the tactile cells, terminating on some of them as end-bulbs and menisci.

Figure 8 is a tactile spot from the palate of the alligator. There is no pigment to confuse the picture and you may have a clearer view of the group of tactile cells in the corium, separated from the epidermis by a narrow strip of corium, and the nerve fibers terminating on and among the tactile cells.

Good illustrations of the microscopic appearance of tactile spots may be seen in the reports of Merkel (1880)⁹ and Hulanicka (1909, 1913).^{10, 11} Pinkus (1927, p. 346)¹² reproduces one of Hulanicka's drawings of a tactile spot of the alligator's tongue, which bears a striking resemblance to the microscopic picture of a pigmented mole.

The question now arises — What possible connection can there

be between pigmented moles of human skin and these pigmented reptilian spots that resemble them so closely? The answer is to be found in the evolution of the hair follicle.

III. THE EVOLUTION OF THE MAMMALIAN HAIR FOLLICLE

In the course of evolution, as the mammal evolved from a reptilian form, the reptilian tactile spots disappeared. As tactile organs they were replaced by hair follicles, which appeared first in mammalian skin.* If we can picture to ourselves a point on mammalian skin where this transformation has not taken place completely, a point where hair follicles have appeared but where reptilian tactile spots have not disappeared, we should have a fair picture of a pigmented, hairy mole. Insert a few hairs in the tactile spots of this alligator and again you would have a good likeness of a hairy, pigmented mole.

The pigmented, hairy mole, then, appears to be a link or transition from pigmented tactile organs of the amphibian-reptilian type to hairy tactile organs of the mammalian type. It offers a picture that is only partly mammalian, the other part being reptilian. In its hair follicles it is mammalian; in its pigmentation, elevation and in the groups of innervated tactile cells in the corium it follows the amphibian-reptilian pattern.

We are well aware of the obscurity that veils the early steps of the evolution of mammalian hair. Animals intermediate between reptile and mammal have largely disappeared or are known only as fossils, of little use in the study of nerve terminals. We are familiar also with the hypotheses by which Maurer (1892, 1895, 1922),^{15, 16, 17} Oppenheimer (1896),¹⁸ Botezat (1914),¹⁴ Broman (1920, 1921),^{19, 20} Preiss (1922),²¹ Plate (1922)²² and others have sought to bridge this evolutionary gap. Discussions and bibliographies may be found in Botezat (1914),¹⁴ Pinkus (1927)¹² and de Meijere (1931).²³ Among these varied and often conflicting opinions the chief supports of our hypothesis stand unshaken. Everyone agrees that the skin of modern reptiles and amphibia contains innumerable tactile spots and that in some manner mammalian skin has succeeded in replacing the reptilian tactile spots with mammalian hair.

* Zoölogists hold that hair is primarily a tactile organ, adapted secondarily to the functions of color and bodily warmth (Plate, 1922, p. 308,¹³ Botezat, 1914, p. 25, 27, 31¹⁴).

Der Spitzmaus

The possibility of the persistence in mammalian skin of nerve terminals resembling amphibian tactile spots needs no lengthy argument, for it actually occurs in one of the shrews. Merkel himself, discoverer of the tactile spot, writes that among the hair follicles on the back of the Spitzmaus (*Sorex pygmaeus*) he saw "nerve fibers ending in groups of cells which lay just beneath the epidermis. These are certainly end-organs but they looked much more like amphibian tactile spots than the customary tactile corpuscles of mammals" (1880, p. 143).⁹

IV. THE PIGMENTED MOLE AS A PHYLOGENETIC TUMOR

The more imaginative of the medical profession have long held a vague belief in the existence of phylogenetic tumors (Bland-Sutton, 1893, p. 493),²⁴ and among phylogenetic tumors they have been especially impressed by the resemblance of hairy moles to the skin of the lower mammals. Pathologists generally had accepted Cohnheim's teaching (1877)²⁵ of the origin of certain tumors in groups of cells that had remained unutilized in the building of the human embryo, when Ribbert (1906)²⁶ pushed some origins farther back and declared that uterine adenomyoma, intestinal polyposis and pigmented moles could not be explained as aberrations in the growth of the human embryo, but that they were easily intelligible as imperfect survivals of structures normal to animals lower in the phylogenetic scale. Mathias (1920, 1922)^{27, 28} came to the same conclusion concerning tumors arising in aberrant pancreas and parotid tissue, noting that such scattered distribution of these glands is normal in earlier evolutionary forms. He proposed grouping such tumors under the name progonoblastoma (*progonoi*, ancestors), and Jendralski (1921)²⁹ described a pigmented mole of the conjunctiva as a progonoblastoma.*

Meirowsky (1926)³⁰ wrote of congenital vitiligo and pigmentation: "Since they correspond so closely with the markings of the lower animals, it follows that in human skin there exist phylogenetically fixed localizations in which a change to nevus formation can occur."

* Mathias (1922)²⁸ announced a forthcoming publication to be entitled: "Zur Auffassung der Naevi als Atavismen im Sinne der Progonoblastom." This paper was never published (personal letter).

Searching in human skin for some vestige of the tactile hairs (sinus hairs) which are widespread among mammals other than man, Henneberg (1915)³¹ thought to find them in pigmented, hairy moles, a view advanced also by Friedenthal (1908),³² by Sklarz (1926),³³ and by Sulc (1926),³⁴ who includes vascular nevi as vestiges of the blood sinuses of sinus hairs. In this group of phylogenetic tumors, Šikl (1932)³⁵ inclines to place two benign epitheliomas of human skin containing mucus-secreting goblet cells, a common feature of the skin of the lower vertebrates but known among mammals only in the skin of the hippopotamus; and Kyrle (1925, p. 253)³⁶ regards human syringocystadenoma as a vestige of the apocrine glands of the lower mammals.

The ideas of this period were well summarized by Maurer (1929)³⁷: "A man is not merely the child of his parents. He is rather the end-product of an immeasurable line of ancestors whose witnesses are the single steps of his embryonic evolution and the many vestiges in his finished form. Such testimony shows that the genes of the impregnated ovum contain the hereditary factors of many different types of animals." *

Back to the Reptile

In explaining the origin of pigmented moles on the principle of phylogenetic atavism all writers without exception have made the same blunder. They forget the nevus cells. From Ribbert onward, they have compared moles in a general way to "the pigmented skin of the lower animals," or more specifically to the hairy tubercles of mammals other than man. But there are no nevus cells in the pigmented skin of the lower mammals or even in their hairy tubercles, whereas nevus cells are an almost constant feature of pigmented moles. Moreover these writers overlooked the nerve fibers. Since the work of Soldán, and especially of Masson, we can no longer hold the simple view that pigmented moles are mere bits of brown or hairy skin inherited from remote mammalian ancestors. When Masson showed that pigmented moles are tumors of the sensory nerves of the skin, that some of the nerves terminate on the nevus

* The phylogenetic sequence of pigmented, hairy moles may be viewed in reverse, making the mole itself the starting point of anthropological studies and working backward, as Samberger (1933)³⁸ has done with naevus acanthosiformis.

cells, and that some pigmented moles contain myriads of meissnerian tactile corpuscles, he obliged the palingeneticist to shift his ground from the study of remote ancestors with pigmented, hairy skins to the study of remote ancestors with pigmented tactile terminals buried in the corium. In so doing we must pass over the entire series of mammals, where no such terminals exist (the end-organs of Merkel's shrew are neither pigmented nor elevated), to ancestors still more remote, the reptiles and amphibia, where pigmented tactile terminals buried in the corium abound. Here at last in the reptilian tactile spot we find a full length portrait of the human pigmented mole, a pigmented elevation of the skin and a nerve coming up from below to innervate a group of tactile cells in the corium.

A phylogenetic theory of pigmented moles must give nerve fibers and nevus cells equal rank with hair and pigmentation. It is true that hair, pigmentation and nerve fibers might represent merely the reappearance in human skin of a patch of mammalian hair, but the innervated nevus cells inexorably carry us farther back, linking that mammalian hair with its evolutionary predecessor, the reptilian tactile spot.

V. ONTOGENESIS

Whether or not we accept a phylogenetic influence in the formation of the pigmented, hairy mole in human skin, we must still seek the immediate fault in the skin of the individual possessing the mole, in ontogenesis. In this search the reptilian aspect of the pigmented mole may prove to be a useful guide. Since the mole is overwhelmingly congenital and often hereditary its ground must have been laid during embryonic life. There is then a certain point in the growth of human embryonic skin when the dipping epidermis and the advancing nerve fibers, which normally coöperate to construct innervated hair follicles, revert to the construction of innervated reptilian tactile organs. One would be inclined to seek this point in a stage of the human embryo when it has not yet diverged greatly from the form and potencies of the reptilian embryo.

Experimental embryology is showing that the various tissues, and in some cases organs, which develop harmoniously to form the individual, are determined very early in embryonic life (Lillie, 1929).³⁹ That is to say, an irreversible change occurs in the nature of the still undifferentiated part which restricts its future capacity for de-

velopment. By such a restrictive change, for example, the cells composing the primitive ectoderm are segregated into those capable of developing into neural tissue and those whose potency is restricted to the production of epidermis. Parts of the epidermis in their turn are progressively restricted to the production of lens, and so on, and finally hair, feathers and other late appearing structures. There are relatively few kinds of such segregates, and the number varies but little throughout the whole vertebrate series.

In the ontogenetic development of the mammal, as compared with that of the reptile, hair appears as a new segregate and simultaneously the tactile spot is lost. If these two organs could be conceived of as segregates from a predecessor equipotential for both, hair being induced by conditions normal to the developing mammalian skin and the tactile spot by conditions normal to the developing reptile, the appearance of the congenital pigmented mole in mammalian skin might be postulated as due to some slight local variation in environment at a critical period. Such a local variation might be of circulatory or neural origin.

The Vascular Nevus

The phylogenetic hypothesis might be extended to vascular nevi. The most primitive amphibia had no lungs; they breathed entirely through the skin. Many salamanders living today have preserved this primitive type of respiration, bearing in their skins a rich capillary plexus that serves as a cutaneous lung. The capillary plexus of vascular nevi may be interpreted as a vestige of the respiratory plexus inherited from that far-off ancestor, the primitive amphibian that breathed through the skin.

SUMMARY

Photomicrographs of silver-stained sections are presented to show that pigmented moles are rich in nerve fibers and tactile cells. End-bulbs of nerve fibers make contact with nevus cells exactly as they do with the tactile cells of the epidermis and of the hair follicles.

Masson's observation of a profusion of structures resembling meissnerian tactile corpuscles in certain pigmented moles is confirmed.

In its elevation, pigmentation, innervation and the groups of tactile cells in the corium, the pigmented mole bears a striking resemblance to the tactile spots of reptiles and amphibia.

In the course of evolution the reptilian tactile spots were replaced by mammalian hair follicles. The pigmented, hairy mole appears to be a link or transition from pigmented tactile organs of the reptilian type to hairy tactile organs of the mammalian type. In its hair follicles it is mammalian, in its pigmentation, elevation and in the groups of innervated tactile cells in the corium it follows the amphibian-reptilian pattern.

Phylogenetic and ontogenetic hypotheses are presented.

For helpful criticism and advice on zoölogical questions and for reptilian material we are indebted to Dr. G. Kingsley Noble of the American Museum of Natural History, to Drs. H. E. Anthony and George G. Goodwin of the same Museum, and to Dr. W. J. Hamilton, Jr., of Cornell University for specimens of American shrews. Dr. Jerome Webster has long taken a friendly interest in these studies, contributing many pigmented moles from his clinic of plastic surgery. To Professor Purdy Stout, the most generous and self-effacing of laboratory directors, we owe many valuable suggestions and choice material.

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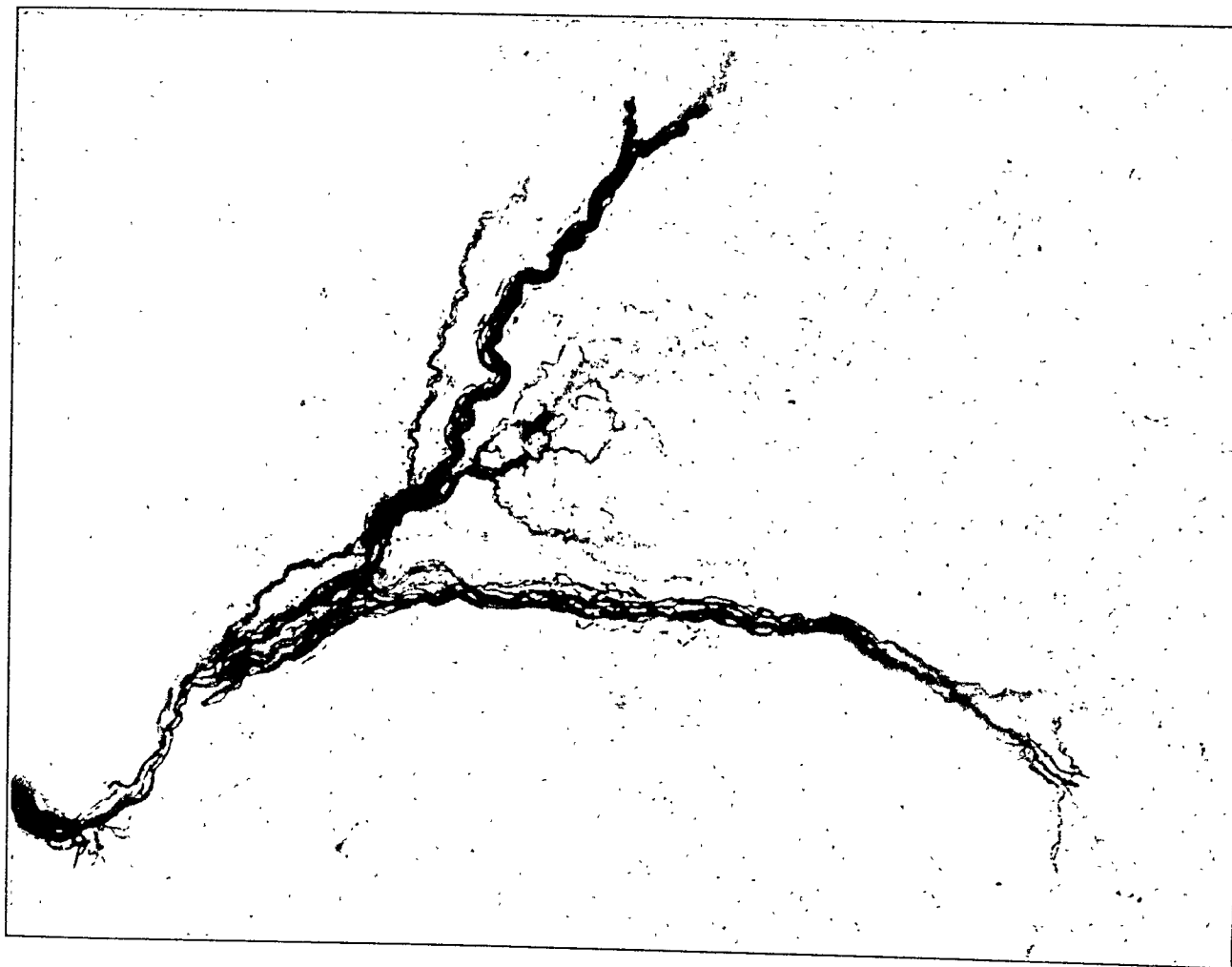
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With the exception of the paraffin section of Figure 4, all tissue was fixed in formalin slightly alkalinized with sodium hydrate or pyridin. Thick frozen sections were stained by Laidlaw's 2 minute silver technique for neurites. This is a modification of Gros-Bielschowsky. Gold toning was omitted. With this technique collagen is usually colorless, cytoplasm colorless to pale yellow, nuclei yellow to brown, and neurites black. The sections are cut very thick, 50 to 75 microns, in order to follow the course of the nerves for long distances. With these black neurites on a colorless or pale yellow ground thickness of the section is no obstacle to satisfactory microscopic examination, although thick sections are not so suitable for photography. Photomicrographs were taken through Wratten filters.

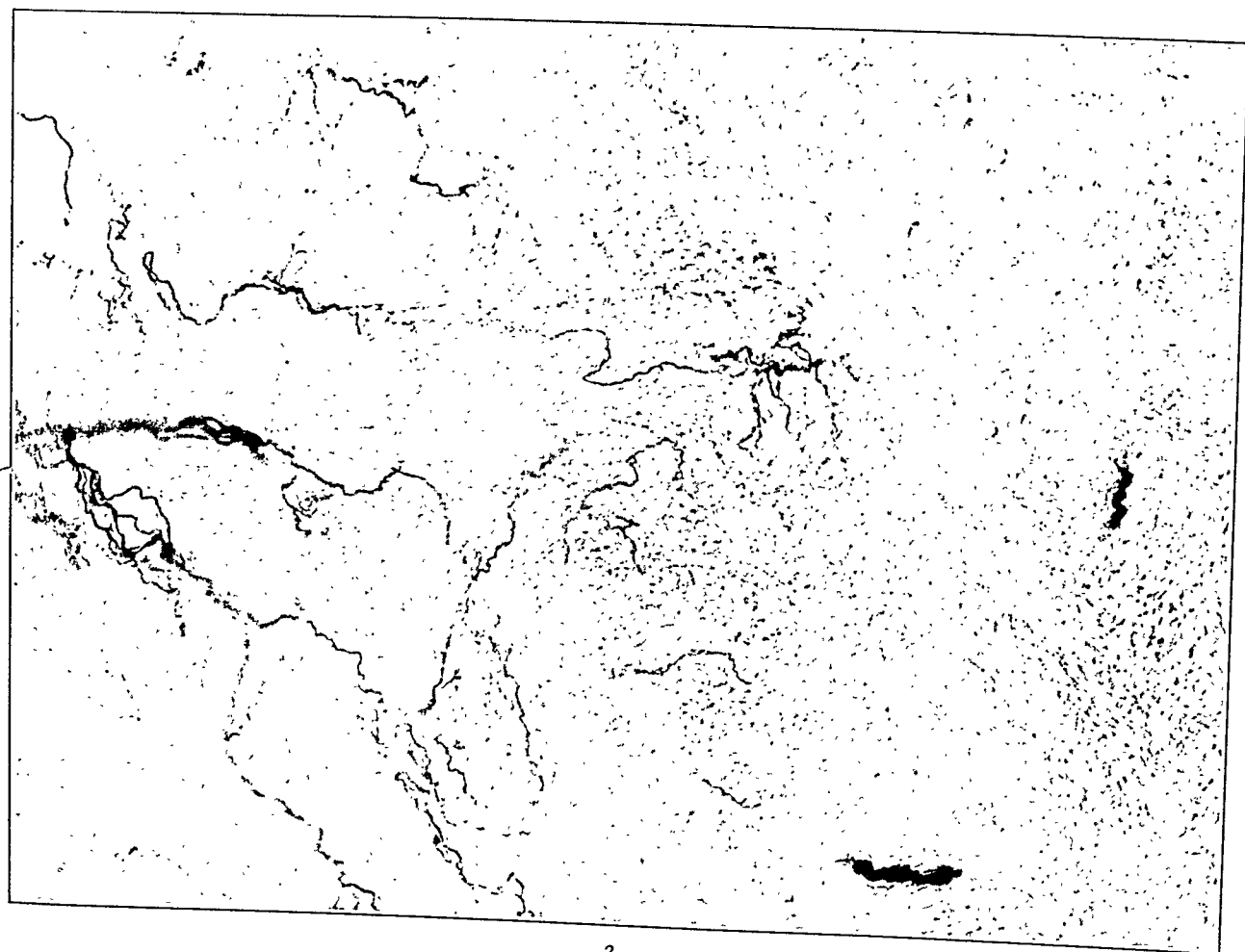
PLATE 133

FIG 1. A nerve trunk branching in the center of a very cellular pigmented mole. The ground is a broad sheet of nevus cells, pale yellow in the section but scarcely distinguishable in the photograph. Just above the crotch of the nerve a short branch goes to the right, soon breaking into fine threads that wind among the nevus cells and end on some of them as tiny bulbs. This is the characteristic termination on tactile cells.

FIG. 2. Distribution of a nerve in a pigmented mole. The entire background is a broad sheet of nevus cells, seen indistinctly in the picture. At the left a nerve trunk gives off many delicate threads that wander among the nevus cells and end as tiny bulbs on some of them. The black figures on the extreme right are short sections of nerve trunks.



I



2

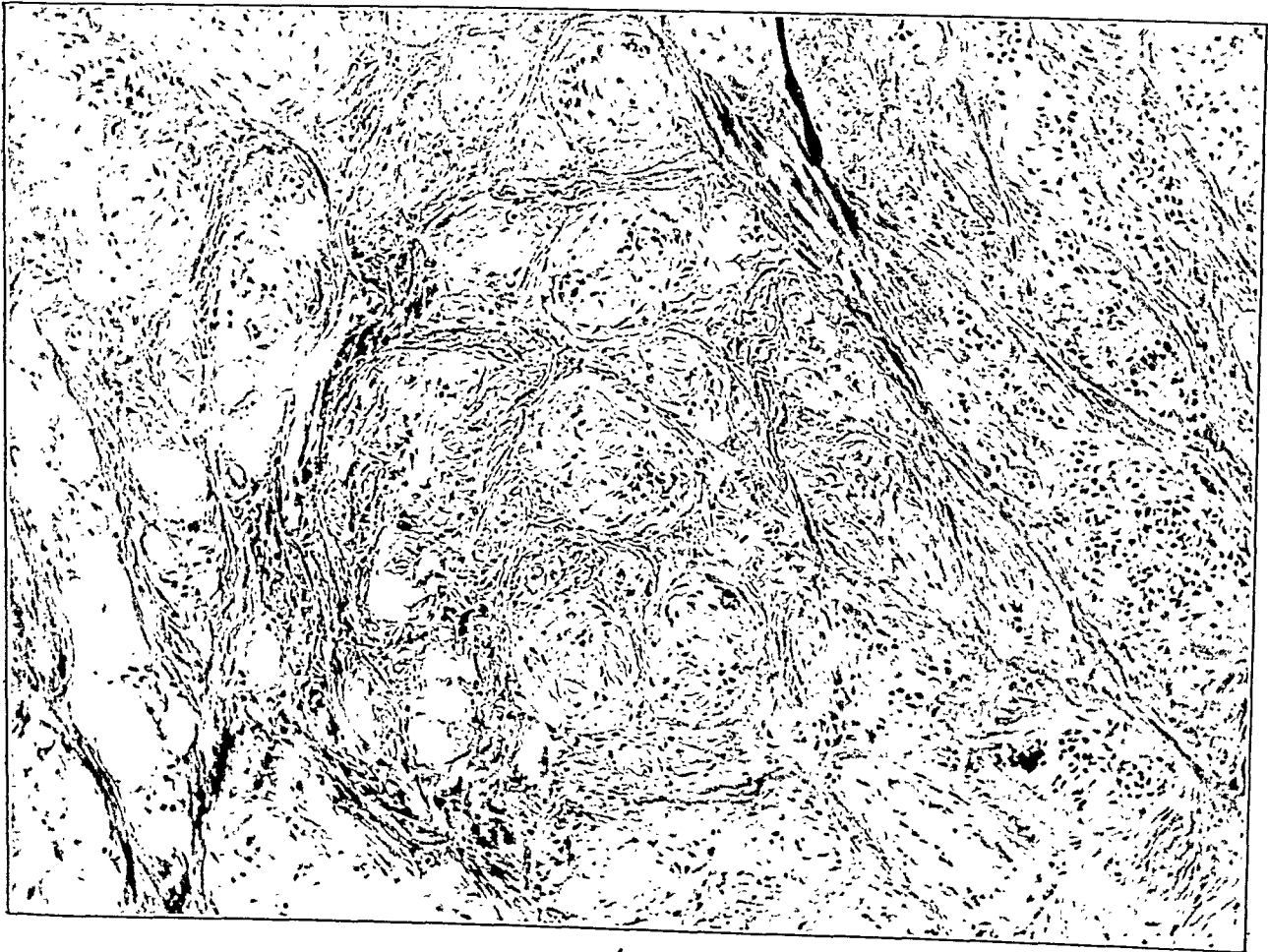
PLATE 134

FIG. 3. Joint distribution of a nerve to a hair follicle and to the surrounding nevus cells. In the right upper corner is a hair in its follicle. The epithelium of the sheath is invisible, colorless in the section. The rest of the picture is a broad sheet of nevus cells. In the center a cross-section of a nerve trunk is seen just at the point where it is dividing. Some branches pass upward and to the right to supply the sheath of the hair follicle. These branches end as tiny bulbs among the epithelia of the hair follicle. Other branches pass upward to the left and downward to the right among the nevus cells. These branches end as tiny bulbs on the nevus cells exactly as the other branches end on the tactile epithelia of the hair follicle. Such joint innervation of hair follicles and nevus cells is very frequent.

FIG. 4. Paraffin section of a pigmented mole from the occiput, fixed in Bouin's fluid. Masson-Mallory trichrome stain. Instead of the usual groups of nevus cells this mole consists almost entirely of round and oval figures, laminated and nucleated, closely resembling meissnerian tactile corpuscles.



3



4

PLATE 135

FIG. 5. From the margin of a pigmented mole. The dark band across the top is epidermis, moderately pigmented but unduly blackened by the silver. Well below the epidermis, running across the colorless corium, is a slender bundle of black neurites, cut by the knife into three sections. The neurites are enclosed in a very thick sheath of cells that blend with the surrounding corium without sharp demarcation. There is no limiting perineurium. This formation is characteristic of neurofibroma of the skin.

FIG. 6. Nerve skein of normal meissnerian corpuscle from the sole of a human foot. The corpuscle lies in a broad, colorless papilla. The irregular black band above it and to the left is normal epidermis. Of the nuclei of the corpuscle, only a few are stained. The nerve skein of the corpuscle is broken into three sections, as often seen in these long corpuscles. The neurites end as bulbs of various sizes in contact with the tactile cells of the corpuscle. In some corpuscles the end-bulbs are enormous. Just below these corpuscles lateral branches often leave the nerve to supply the adjoining epidermis.



5



6

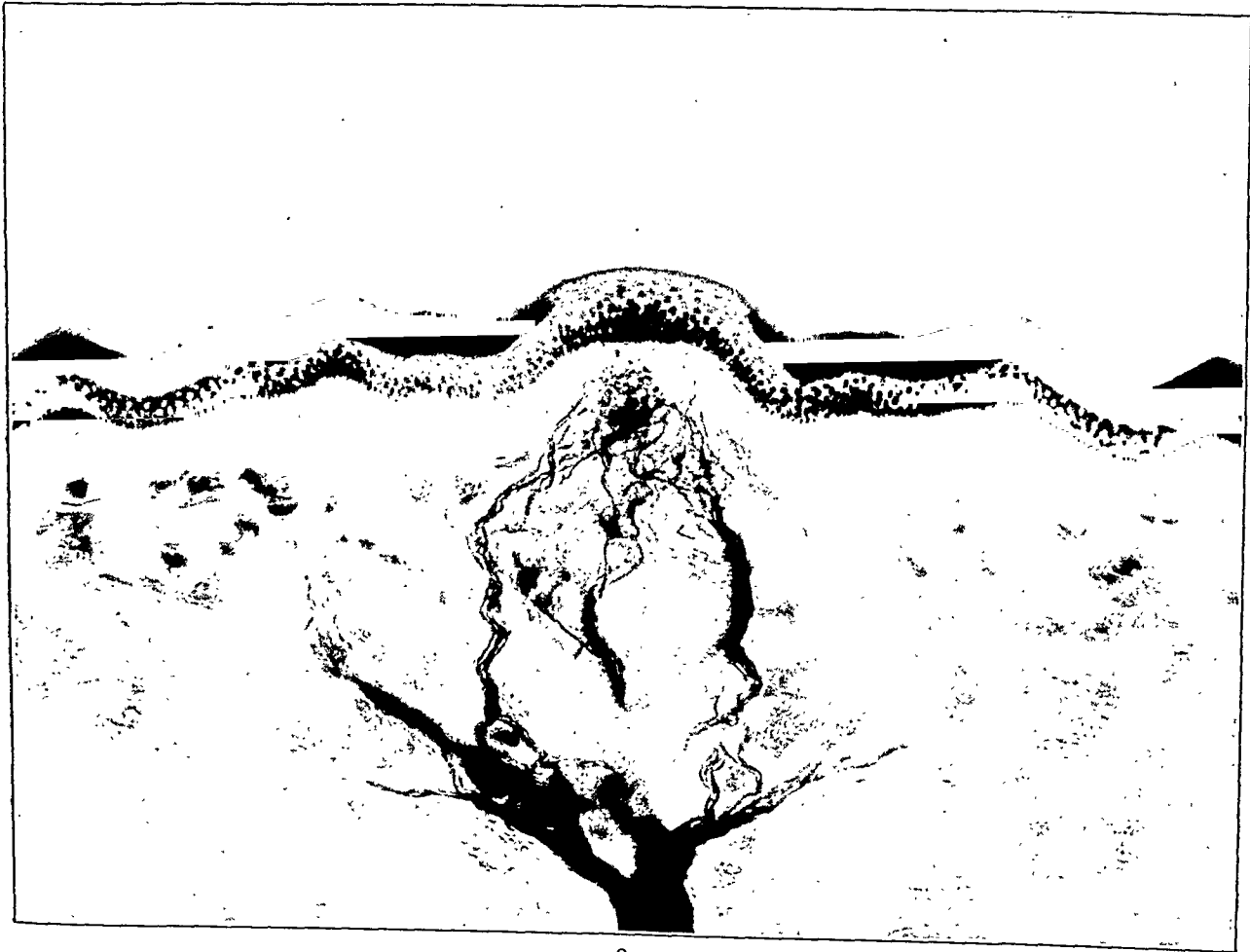
PLATE 136

FIG. 7. Vertical section through the black tactile spot of an alligator's jaw. Note the conical elevation of the derma with its group of tactile cells. In the natural state the pigmentation is limited to the elevation; in the picture the whole epidermis has blackened in the silver. The black figures in the lower and middle areas are nerve trunks from which many branches ascend to supply the tactile spot. Here they break into fine threads that wind among the tactile cells and end as tiny bulbs or menisci on some of them. The group of tactile cells constituting the spot lies just beneath the epidermis. Between the tactile cells and the epidermis there is a narrow strip of corium which does not show in the picture, the section being very thick. Some nerve fibers continue upward across this strip and enter the epidermis. According to Plate (1924, pages 61 and 63), the meissnerian corpuscle of human skin is a direct evolutionary form of these reptilian tactile spots.

FIG. 8. Tactile spot from the palate of the alligator. There is no pigment, although the stratum corneum of the epidermis has blackened in the silver, except over the tactile spot. Beneath this rounded elevation there is a group of tactile cells with many delicate nerve fibers winding among them and ending on some of the cells. Between the tactile cells and the epidermis there is a broad band of colorless corium. Some of the nerve fibers cross this space to enter the epidermis. Below a large nerve trunk branches upward, sending three bundles to supply the tactile spot.



7



8

THE CYTOLOGY OF THE GLIOMA GROUP; WITH SPECIAL REFERENCE TO THE INCLUSION OF CELLS DERIVED FROM THE INVADDED TISSUE *

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INTRODUCTION

In the histological study of the material to be described in this paper the writer was impressed by certain difficulties in identifying individual cells, difficulties that many writers have ignored or have touched on but lightly. The chief source of these difficulties was the persistence within the tumours of included normal brain elements or of reaction forms derived from them. The included non-neoplastic elements were most frequently astrocytes or their derivatives, and less frequently nerve cells and fibres.

The study of these included elements, though at first sight of little importance, is nevertheless essential to correct interpretation of the histogenesis of the tumours. The nature of certain tumour cells may be suggested by the presence of more differentiated forms in their vicinity, and it is imperative, therefore, to determine whether these forms are merely included non-neoplastic elements or whether they are indeed tumour cells derived from or producing the less differentiated cells.

This paper, the aim of which is to study the nature and history of the individual cells rather than of the tumours as such, is divided, therefore, into two parts. In the first, those cells that appear to be truly neoplastic are described and discussed. In the second, certain observations on the reaction of the invaded tissues are recorded, and the relation of this reaction to inclusion forms within the tumour zone is discussed.

In order to do this it is necessary to accept some form of tumour classification as a basis. The Bailey-Cushing classification of 1926¹ is admirably suited to this purpose, as it embraces names that have been widely accepted in the English-speaking world. This classification will probably be considerably modified in the future. It pos-

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sesses certain minor disadvantages, but at present it has the great advantage of being the common language of many neurologists, neurosurgeons and pathologists. The value of its conception can hardly yet be appreciated. Based on an unrivalled series of cerebral tumours it forms a great landmark in the history of the study and classification of the glioma group.

The specific impregnation methods of Golgi, Bielschowsky, Cajal, and del Río-Hortega were employed by Bailey and Cushing in their study of gliomata. In 1926 they produced their monograph. In it they adopted a classification based on histogenesis, and divided these neoplasms into fourteen categories. The majority of the types had been previously described, but to them they added the important, if uncommon, group of the oligodendrogliomata.

They sought in each of their large series of tumours representatives of the various embryonic or adult types. Where a tumour occurred which seemed to contain a predominance of some particular cell, they named that tumour accordingly. The nature of these cells was deduced from a study of their form, their affinity for various stains, and the appearances of the more adult forms in their vicinity.

Such a classification based on embryogenesis has been applied to tumours elsewhere. It is doubtful if in a pure form it is entirely satisfactory. There are also certain features of this particular classification that require some consideration.

1. From Bailey and Cushing's presentation of the subject the impression is gained that these authors assume that tumours arise from cells that have suffered arrested development. Bailey has recently disclaimed his belief in such a theory² and states that the most he has done is to give names to the tumours chosen from the histogenesis of the nervous system, because the cells of the tumours seem to resemble in their structure these embryological forms. In the classification this is not made clear. The tumour cells are usually described, with the exception of those of the spongioblastoma multiforme, as *being* such cells, not as *resembling* such cells.

At the same time the theory of embryonal rests and the origin of tumours from heterotopias is seriously discussed. There is undoubtedly a certain amount of evidence to suggest that errors of development may be associated with cerebral tumour formation. Thus the medulloblastoma of the cerebellum has been noted to occur in twins. Retinal neurocytoma, a similar tumour, is frequently bilateral and

familial. Neurofibromatosis may be associated with gliomata in the region of the optic chiasma or even, as in a case described by Schnyder, with a circumscribed glioma in addition.³

In Lindau's disease various developmental defects are associated at times with a cerebellar haemangioblastoma, while the cerebellar ganglioneuromata have been noted to occur in association with various developmental deformities. In this series an oligodendroglioma was associated with numerous small subependymal nodules, although these could not be said to be truly neoplastic.

The frequency of the occurrence of tumours about the fourth ventricle of young subjects is also held to indicate an origin from cell rests, as in this area heterotopias of various sorts have been observed.

When we consider the tumours of later life, and in particular the so-called spongioblastoma multiforme, we find little evidence to support such an origin, although this is contrary to the view held by Globus.⁴ Bailey and Cushing were always uncertain of the origin of this tumour as they had observed its differentiation from the protoplasmic astrocytoma. Now Bailey² speaks of its constituent cells as anaplastic cells. He prefers the non-embryogenetic name of glioblastoma.

The cells of glioblastomata possess every characteristic peculiar to anaplastic tumours, that is, to tumours composed of undifferentiated cells derived from more adult elements. Polarity of the cells is largely lost, and they possess abundance and abnormality of mitotic figures. Moreover, such tumours have been observed to be derived from such well differentiated tumours as the astrocytomata, this change in characteristic not uncommonly following operative interference.

If we are confronted with a section containing astrocytes and less differentiated forms, and we are assured that such astrocytes are not included, there are two possibilities, *viz.*, the astrocytes may be differentiated from the less differentiated forms, or the less differentiated forms may, by the process of anaplasia, be derived from the astrocytes.

Roussy, Lhermitte and Cornil have clearly recognised the difficulties of the Bailey-Cushing classification. Roussy⁵ remarks that they would prefer to class tumours of the nervous system according to their objective structural aspects and their predominance, with-

out seeking to draw from morphology deductions of an embryological or histogenetic order, which are far from being demonstrated.⁵

Ewing,⁶ in speaking of tumour classification in general, summarises the situation: "The generally accepted plan of classification and terminology which is based on histology, modified as much as possible by histogenesis, is a natural product which has become very firmly established, and probably deserves to prevail against the varying prominence of embryology, chemistry and etiology."

2. Another difficulty of this classification arises when we examine certain individual tumours. Even if we believe that the cells of a tumour are embryonal in type, it is not always possible to be sure which cell type they represent. This is exemplified by the unipolar spongioblastoma of Bailey and Cushing. As the name suggests, they regard the cell type of this tumour as a unipolar spongioblast or precursor of the astrocyte line. Josephy,⁷ however, described this tumour as a *neurinome centrale*, on account of its striking resemblance to the common auditory nerve tumour. Roussy and Oberling⁸ believe it to be composed of oligodendroglial elements, while Courville^{9,10} sees in it an immature ganglion cell tumour.

The so-called medulloblast is another difficulty. Bailey and Cushing find glial and neuronc elements within medulloblastomata and so liken the cell type to the hypothetical undifferentiated cell of Shaper, which may differentiate along either line. Penfield¹¹ does not find neuroblasts and considers the medulloblast to be an apolar spongioblast. Roussy and Oberling⁸ would prefer to use the term neurospongioma for the tumour, as they have found a fine groundwork of neurofibrils in certain of their examples. Ewing⁶ classes it under the neurocytomas. Ribbert¹² describes a tumour that may be of this type with unusually well marked rosette formations and likens it to the rare suprarenal neuroblastoma.

3. In the Bailey-Cushing classification it is usually assumed, as already stated, that more adult forms occurring with less differentiated elements are derived from the latter. Even if this could be accepted, we are yet faced with another practical difficulty. A glioma is a glial formation invading glial tissue, and it may be extremely difficult to determine which are the invading cells and which the cells of the invaded tissues. It must be remembered that the glioma is usually only locally malignant, and, except in a few implanted secondary tumours recently described by Russell and

Cairns,^{13, 14} we have few examples of the appearance of such tumours in an unrelated tissue.

This point is of importance as many authors base their view of the nature of the more immature forms on the undoubted histological nature of the adult forms. It is true that the gap between is always said by them to be bridged by intermediate forms, but even this appearance at times may be misleading.

In my experience included astrocytes may mimic spongioblasts, astroblasts and giant cell forms, and included neurones may present the appearance of neuroblasts. Only a close study of the tumour edge and the neighbouring invaded area will solve the mystery of many of these forms. A small biopsy portion removed at operation may be entirely misleading.

This criticism does not suggest that many of the excellent names used by these authors should be discarded. Time and additional study will undoubtedly result in certain alterations that seem more appropriate. In the process of modification there is no necessity to keep to names with an embryogenetic significance. By doing so one is apt to convey an incorrect idea of the nature and evolution of the tumours.

The Bailey-Cushing classification has been of the greatest utility to the neurologist and the neurosurgeon. It has focussed attention on tumours of heterogeneous appearance and has resulted in the recognition of definite clinical types of tumours, with definite natural histories and methods of treatment.

Since the original subdivision into fourteen categories simplifications have been introduced. Minor differences of structure, as, for instance, the distinction between ependymoblastoma and ependymoma, have been discarded for practical purposes. In a later publication Bailey¹⁵ has reduced the categories to ten. Thus the two types of astrocytoma have been fused (Cushing,¹⁶ Penfield¹⁷), and the pineoblastoma has been included with the medulloblastoma.

Roussy and Oberling⁸ have recently introduced a new and instructive classification. Its main distinction is that certain cells, regarded by Bailey and Cushing as embryonal, are considered by them to be of an adult nature. The apparent embryonal form indicates to them a degenerative change. Thus they describe several varieties of glioma, a term applied in their classification to any tumour composed of adult astrocytes. The differences in appearance are

described in more detail than is usually given, and although they accept most of the tumour types described by Bailey and Cushing they discard one, the astroblastoma, as representing a degenerate form of an astrocytoma. In addition, they are not entirely in agreement as regards the actual histology of other forms. This atlas is an extremely instructive and valuable work, and should be read in association with the Bailey-Cushing classification. Certain features are discussed later in this paper.

The simplest classification proposed is that of Carmichael.¹⁸ It is essentially a practical subdivision into the more adult and the grossly anaplastic forms. The first he would call the spongioblastic group and the second the astroblastic.

Table I, somewhat modified from Brain and Strauss,¹⁹ with additions, shows the relation of the many names applied to the gliomata.

Text-figure 1 represents the scheme of evolution of certain elements of the central nervous system, and will serve as a key to names commonly used in describing the histology of these tumours.

In this paper cells that are not truly neoplastic and are the residue of, or derived from, the invaded tissue are for the sake of convenience described as inclusion cells.

METHODS

The material used has been a series of 120 intracranial tumours, including 70 gliomata, mainly obtained in the postmortem room. Most of these were obtained recently, but a number of valuable specimens have been obtained from pathological museums. The impregnation of these old specimens was usually extraordinarily good, except in the case of the gold stains.

Various areas of the tumours were studied, and in particular the growing edge.

The majority of specimens were preserved in formalin. The method of Davidoff²⁰ was occasionally used for reimpregnation with Zenker's fluid. Isolated examples were put directly into Zenker's fluid or into formol bromide. Some of the older specimens may have been originally preserved in spirit, but were in dilute formalin at the time of examination.

Paraffin sections and frozen sections were used together in most cases. The methods employed as the occasion demanded were:

TABLE I

Bailey and Cushing	Bailey (1927)	Roussy and Oberling (1931)	
1. Medulloblastoma	1. Medulloblastoma	Neurospongione	Neuroblastoma (Wright)
2. Pineoblastoma			Neurogliocytome embryonnaire (Masson)
3. Neuroepithelioma	2. Neuroepithelioma		Glioma sarcomatoides (Borst)
			Neuroépitheliome (Flexner)
			Retinocytome à stephanocytes (Mawas)
			Blastome ependymale (Marburg)
4. Spongioblastoma multiforme (glioblastoma)	3. Spongioblastoma multiforme (glioblastoma)	Glioblastome	Spongioblastoma multiforme (Globus and Strauss)
			Glio-sarcoma (Ewing, Borst)
			Gliome à petites cellules (Masson)
			Gliome polymorph (Roussy, Lhermitte and Cornil)
5. Pinealoma	4. Pinealoma		Pinealoma (Krabbe)
			Chorioma (Askanazy)
			Compound pineal gland type (Strong)
6. Spongioblastoma unipolare	5. Spongioblastoma unipolare	Oligodendrocytome fasciculé	Neurinome centrale (Joseph-Macpherson)
7. Astroblastoma	6. Astroblastoma	(Included in astrocytomas)	Neuroblastoma? (Greenfield)
		(a) Ependymocytome	
8. Ependymomas	7. Ependymoma	(b) Ependymoblastome	Ependymal glioma
9. Ependymoblastoma		(c) Ependymogliome	Glio-ependymome (Masson)
10. Astrocytoma fibrillare	8. Astrocytoma	Astrocytome	Astrocytoma (Ewing)
11. Astrocytoma protoplasmicum			Sternzellen (Stroebe)
12. Oligodendroglioma	Oligodendroglioma	Oligodendrocytome	Gliome à petites cellules rondes (Roussy, Lhermitte and Cornil)
13. Ganglioneuroma	Ganglioneuroma		Neurogliome ganglionnaire

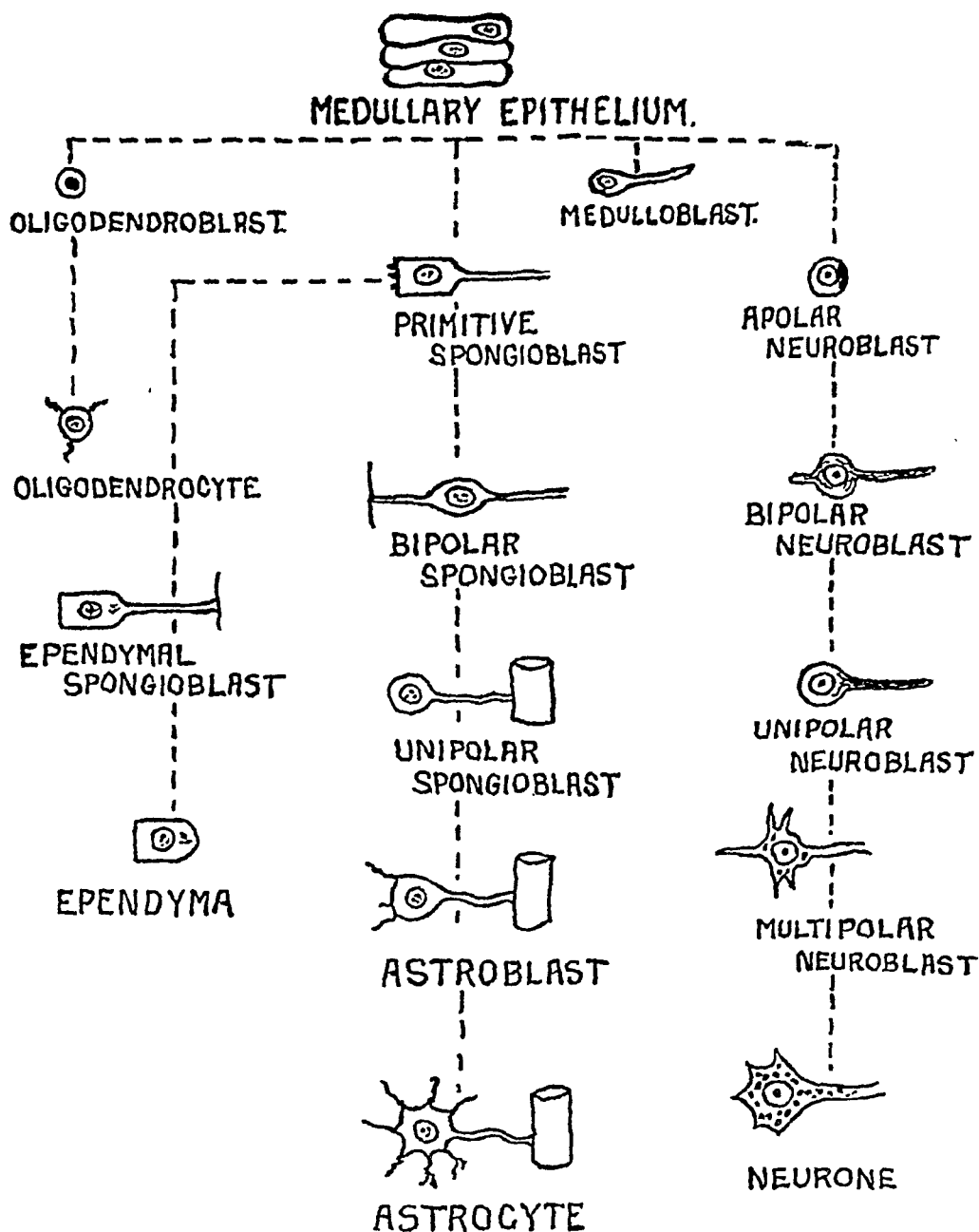
1. Paraffin sections: Haematoxylin and eosin.
 Haematoxylin and Van Gieson.
 Mallory's phosphotungstic acid haematoxylin.
 Weigert's iron haematoxylin.
 Nissl's method.
2. Frozen sections: Haematoxylin and eosin or Van Gieson.
 Cajal's gold sublimate stain — after formol bromide
 or more usually following the Globus-Penfield tech-
 nique.^{21, 22}
 Cajal's pyridine silver method.
 Hortega's fourth variant.
 Hortega's method for oligodendroglia and microglia fol-
 lowing the Cone-Penfield modification.
 Bielschowsky's method for frozen sections.
 Weigert's medullary sheath stain.

In ordinary postmortem material, as received in the Baker Institute, it was soon realised that the metallic stains were only specific in a comparative sense. In this regard gold sublimate was perhaps the most satisfactory and rarely provided any impregnation of cells less mature than those at a well formed polar stage. Included astrocytes and their modifications were, however, usually clearly impregnated. The very old tumours, with occasional exceptions, did not impregnate satisfactorily. One tumour nearly 30 years old gave a fair impregnation, while certain more recent ones were unsatisfactory.

The silver pyridine stain of Cajal was originally considered specific for neuroblastic forms, and in the first classification deductions were made as to the nature of cells based on this specificity. Bailey later discovered that this was not entirely so. In the experience of this laboratory normal neuroglia was rarely impregnated, although any large hypertrophic forms, whether neoplastic or about the tumour edge, might be. Many astroblastic forms, as well as polar and asteroid forms, were at times densely impregnated. The older the material, the less specific this stain seemed to be. The polar cells of an ependymoma, which had been preserved for 37 years, were densely impregnated.

The combined method for oligodendroglia and microglia astonished the author by impregnating some of the oligodendroglial cells in an undoubted oligodendroglioma that had been preserved for 28 years.

The silver carbonate method often gave excellent impregnation of nerve inclusions, as well as astroblastic and asteroid forms, particularly if the sections were deliberately overstained.



TEXT-FIG. 1

Illustrating the terminology of embryogenesis of the cell elements of the central nervous system, as applied to cells of somewhat similar appearance and staining reaction in the gliomata.

PART I

MEDULLO-EPITHELIOMA

It is appropriate to commence the discussion of the individual tumour groups by considering the very rare medullo-epithelioma. Bailey and Cushing place this at the head of their classification, as it represents to them the arrangement of the medullary epithelium, the first indication in the embryo of the developing nervous system.

Such a tumour consists of bands and folds of columnar cells, with oval nuclei, possessing well marked external and internal limiting membranes. They have observed two examples, one arising in the postinfundibular region, and the other in the midbrain. Mitotic figures were numerous, and each tumour presented appearances suggesting considerable malignancy.

The only positions where the embryonic formation of the medullary epithelium is retained as an adult structure are in portions of the floor and the roof palate of the third ventricle, and in the epithelium covering the pars ciliaris retinae. Tumours that arise in these regions may be expected to reproduce something of the structure of the tissue of their origin. As this tissue is no longer an embryonic but a fully differentiated tissue, it should not be expected to reproduce the full range of cells, neuronc and glial, for which the medullary epithelium is responsible. It should be expected to give origin to tumours, some of slow and some of rapid growth, reproducing more or less accurately its own structure, much as does any other adult tissue. The analogy with medullary epithelium is misleading.

In this series a small defined tumour about 1 cm. in diameter was encountered in the midline of the infundibular region. Its consistence was firm, a small cyst occupying its centre. Section showed an arrangement of columnar cells presenting somewhat the appearance of the medullo-epithelioma (Fig. 1). Mitotic figures were not observed, its appearance being essentially non-malignant.

This group of tumours in the cerebrum is extremely rare and no definite opinion can yet be expressed concerning them.

The retinal examples have been recently summarised by Grinker²³ and may be studied in his paper.

NEURO-EPITHELIOMA

Flexner²⁴ in 1891 described a tumour of the retina, for which he chose the name neuro-epithelioma. Other examples have been described, and they have been recently epitomised by Grinker.²³ He adds a further example which he has studied by the full modern histological technique.

The tumour cells tend to arrange themselves into true rosettes, with well defined lumina. Cilia may be observed projecting into the cavities and blepharoplasten are usually seen in the neighbourhood of these cilia. The outer extremities of the cells stream into the neighbouring tissue as tail-like processes which may be attached to neighbouring vessels. The whole form and general arrangement strongly suggest the arrangement of the primitive spongioblasts into neuro-epithelium. Certain non-malignant excrescences of similar structure may appear in relation to the senile ciliary body, or even in infections of the same region, and the resemblance of these cells to normal rods and cones, themselves derived from primitive spongioblasts, has been commented upon.

Tumours of this nature are extremely rare in the brain, no example occurring in the original Bailey-Cushing series. They have been observed in the brain, in the spinal cord, and in the sacrococcygeal region.

Bucy and Muncie²⁵ have recently reported a tumour of the cerebellum which appears to be of this nature. They accept only five other examples, one occurring in the cerebellum (Naeslund's case), one in the frontal lobe (Bailey¹⁵), one near the sylvian aqueduct (Roussy, Lhermitte and Cornil⁵), one in the left cerebral hemisphere (Ribbert¹²), and an unpublished case of Cushing's occurring in the fourth ventricle. They classify other reported examples, with one possible exception (Nyematsus' case), as being ependymomata or ependymoblastomata.

It must, however, be realised that adult cells of the appearance of the primitive spongioblast occur in such regions as the anteromedian fissure, the posteromedian septum of the spinal cord in the median raphé of the hind brain, as well as in the rhombic lip of the fourth ventricle.²⁶ Also very similar cells may be observed in the ependymal lined clefts of the ependymoma (Fig. 2). The distinction then be-

tween the neuro-epithelioma and the ependymoma may be more apparent than real.

For a tumour to be composed of true neuro-epithelium, as opposed to an adult structure of similar appearance, one would expect, as Roussy and Oberling suggest,⁸ evidence of differentiation into spongioblastic and neuroblastic forms. This is not usually evident.

Many of the reported cerebral neuro-epitheliomata do not suggest any great evidence of malignancy. Even those of the retinal type, although showing more evidence of mitotic division, are among the less malignant tumours of this region.

MEDULLOBLASTOMA

This very characteristic tumour occurs predominantly in the mid-cerebellar region in childhood, but may occur in the cerebrum and in adults. The typical cell is small and strikingly uniform in appearance (Fig. 3). In paraffin sections the cytoplasm is indistinct, but in preparations stained by the supravital technique it is very clearly stained and delimited.²⁷ The nucleus, which is round, oval or occasionally elongate, possesses a dense chromatin network and may show mitotic figures. Such cells at times arrange themselves in rosette formation; the cytoplasm may be carrot-shaped, with a tail streaming toward the centre of the rosette.

Bailey and Cushing named this cell a medulloblast, on account of the occurrence of both spongioblasts and neuroblasts within these tumours. A capacity to differentiate in each direction suggested to them the theoretical medulloblast of Shaper, which is credited with this ability.

At a somewhat later date Cushing²⁸ revised this description. The cerebral medulloblastomata were found to occur at a later period of life, to be slower in growth and to have differentiated more fully. It is in this type that most of the described neuroblasts and spongioblasts occur, but Bailey¹⁵ finds cells that he believes to be neuroblasts in the cerebellar type.

Roussy and Oberling⁸ liken the appearance of this tumour to the neurospongium, that is, to the appearance of the primitive neural epithelium, when the germinal cells have separated and are in active subdivision. The fine fibrillary network so common is to them a network of neurofibrils. They find neuroblasts and their progeni-

tors to be constituents of these tumours and distinguish two forms, a *neurospongiome essentiellement neuroblastique* and a *neurospongiome neurospongioblastique*.

Penfield¹¹ finds spongioblasts, but no neuroblasts. As a medulloblast has never been observed in normal histology he considers this cell an apolar spongioblast.

There is a great likeness of this tumour to one tumour described as glioma of the retina. When the latter invades the cerebrum, as it did in a recent case reported by Cairns and Russell,¹⁴ the appearance seems to be entirely similar. Both of these tumour types usually occur in the early period of life.

The polar forms that occur in this condition are interesting (Fig. 4). They are common in the cerebral examples and their extremities fray out into a fine fibrillar substance. Cairns and Russell report a case where polar cells occurred in the cerebellar type. The processes were clearly stained with Mallory's stain, thus suggesting their relation to the astrocyte series. The history of this tumour was unusually long, covering a period of 6 years. However, in two cerebellar examples recently examined in this laboratory, in which polar cells predominated, the courses were exceedingly brief.

A cerebral example occurred in this series in a child. The growing edge presents two distinct appearances, one corresponding to the ordinary appearance of the medulloblastoma, and the other to the polar type. In each of these areas mitotic figures are equally abundant, and there is nothing to suggest that the polar cells have differentiated from the apolar.

The polar forms at the growing edge show nuclei of almost a cigar shape, the cells being disposed in clumps and often presenting an appearance of palisading. The marked fibrillary formation separating these clumps may be distinguished clearly in haematoxylin-eosin preparations. From each clump streams a band of parallel fibrils, which are interwoven with similar strands from other areas. These cells present no suggestion of being neuroblastic, and seem more allied to the astrocytic series. At the same time, the description of them as spongioblasts seems hardly applicable. A similar tumour is figured in Roussy and Oberling's atlas.

Eight cerebellar cases were examined, six occurring in young children and two in adults. Considering that the average life in Cushing's cases was about 1 year from onset, three of these cases

presented unusually long histories. In one, a child 11 years of age, signs of internal hydrocephalus had been present for over 5 years. The history in the second case, in which dissemination over the cerebrospinal axis was wide, extended over $3\frac{1}{2}$ years, and that of the third for over 2 years. In the case with the longest history mitotic figures are few and polar forms are rare. No spongioblasts or neuroblasts are observed, but there is a remarkable preservation of nerve fibres in the tumour substance. There is also a well marked astrocytic stroma, without any suggestion of intermediate forms between astrocyte and medulloblast. In the third case a similar appearance is observed, but more mitotic figures are present. The remaining examples, with one exception, are much more highly malignant and mitotic figures are numerous. In one example the nuclei frequently present a single large nucleolus and a comparison with the nuclei in the neighbouring granular zone of the cerebellum shows a remarkable similarity. This type of nucleus is, however, not uncommon in rapidly growing tumours. Moreover, silver impregnation shows no impregnation of the intercellular network, although climbing and mossy fibres in the neighbouring cerebellar zone are well impregnated.

There is no evidence in these tumours of the occurrence of neuroblasts, even in the more slowly growing types where this might be expected to occur, but there is a certain amount of evidence that such tumours may evolve forms suggestive of the astrocyte series.

There is always considerable difficulty in determining which cell these small-celled embryonal tumours represent (Ewing⁶). My limited experience somewhat favours the view held by Penfield. It must be admitted that a slide of the small-celled embryonal tumours of the kidney could easily be transposed for a medulloblastoma without detection.

GENERAL CONCLUSIONS

1. The so-called medulloblast is possibly a cell of embryonal type, according to the definition of Ewing.⁶ Exactly where it stands in histogenesis could not be determined by any observation made in this series.

2. At times it assumes an elongate form with numerous fibrillae. Although this usually indicates a less malignant tumour, it does not necessarily do so, as some forms may show active mitosis. It may

indicate a tendency to form an astrocytic type of cell of polar form.

3. The cell type is usually very constant. Such cells may be in active subdivision, or again, the tumour may be comparatively slow in growth, as shown by little evidence of cell division.

4. No evidence of the presence of neuroblasts was noted in these tumours.

SPONGIOBLASTOMA MULTIFORME — GLIOBLASTOMA — GLIOSARCOMA

This very common tumour occurs predominantly in the cerebral hemispheres in the middle decades of life. The older name, gliosarcoma, was applied by Stroebe under the belief that the vascular hyperplasia participated in the tumour process. Ewing, with Lubarsch and Adami,⁶ still finds no real objection to the name.

The name spongioblastoma was introduced by Strauss and Globus²⁹ and Ribbert¹² in the same year. The former authors, in a later paper,³⁰ described sixteen cases and presented most of the essential cell elements to be found in these tumours.

The name was accepted by Bailey and Cushing, although Bailey¹⁵ at a later date wrote that they did so with some diffidence and would prefer the name glioblastoma.

Penfield objects to the name glioblast, as there is no such cell in normal histology. One might suggest that this is *not* a *disadvantage*, as these cells are not normal cells but anaplastic tumour cells. It seems better to use a general name, rather than one employed in normal histogenesis.

The glioblastoma possesses every feature that Ewing regards as necessary to discriminate an anaplastic from an embryonal tumour. It is extremely cellular, departs in type from the cells from which it is derived, mitotic figures are abundant, and areas of cells are usually present which have entirely lost their polarity.

Tumours of this type have been observed to arise from tumours regarded as astrocytoma (Tooth,³¹ Globus and Strauss,³⁰ Bailey and Cushing¹). Globus and Strauss found evidence of tuberosc sclerosis in three of their cases, and multiple tumours have also been reported. It is probable that the ordinary clinical type is derived from adult elements.

Tumour mixtures are frequent; areas of astrocytoma, ependymoma, astroblastoma and other varieties are encountered. In this series areas of glioblastoma occurred in association with formations of the nature of astrocytoma, astroblastoma, ependymoma, polar spongioblastoma and again in a tumour that was present in the position of the pineal gland. Bailey and Cushing appear to assume that the more adult cells are differentiated from the less differentiated. This assumption must be accepted with considerable reservation. In many of my cases the appearance was such as to suggest that a more differentiated tumour had become anaplastic.

A large tumour was encountered occupying the lateral ventricle of a man 65 years of age. The structure in parts is undoubted ependymoblastoma. *At one extremity this embryonal appearance is entirely lost, the tumour has assumed the anaplastic appearance of the glioblastoma and has become adherent to the tentorium in which it was actively growing.* In the midline of the cerebellum another tumour of undoubted ependymal nature occurred, but without any suggestion of anaplasia.

On the other hand, certain tumours included in this group do not present such marked evidence of anaplasia. One tumour is composed almost entirely of small unipolar cells taking the gold stain intensely. Mitotic figures are not evident, and the general appearance is that of three tumours described by Carmichael¹⁸ as spongioblastoma unipolare. Possibly this group should be separated from the glioblastoma group. The name spongioblastoma unipolare would have been admirable if it had not been already applied to an entirely dissimilar tumour.

If all the forms encountered within this tumour group were described we should have a description of the majority of cells occurring in the gliomata as a series.

Before such a description is attempted it would be well to mention briefly a very constant feature, the extraordinary proliferation that may occur in the walls of the vascular channels. This hyperplasia involves both endothelium and adventitia, but it is the remarkable multiplication of the endothelial cells that is most characteristic. Stroebe, as previously noted, regarded this change as part of the tumour process, and Penfield¹⁷ has recently expressed the view that it may yet prove to be the essential part of the tumour. Certainly the endothelial proliferation may be observed well beyond

the tumour edge. However, at times a somewhat similar appearance may be noted outside the edge of a metastatic carcinoma nodule. Cairns and Russell¹⁴ have also found similar changes in the vessels of the meninges, where a fragment of glioblastoma had become implanted and was in active growth.

The following cell types, which appeared to be of a true neoplastic nature, were noted:

1. The most constant cell type is of rounded, ovoid or irregular shape. The protoplasm is usually ample, the nucleus clear and vesicular, while mitotic figures are frequently encountered. The size of nucleus and cell is extremely variable, and all transitions occur between such cells and giant cells of great size (Text-fig. 2A). It is apparent from the description of this cell type that it might fit equally well an anaplastic cell of non-glial origin. This is the common and characteristic cell. It may be the only cell type present, and possesses no specific staining properties.

2. Giant cells are usually present. In one tumour they form the predominant cell. Some are of enormous size, and possess either one large single nucleus or numerous nuclei containing various atypical arrangements of the chromatin. These cells again possess no properties peculiar to glial cells. It is not unusual to find a large chromatic sphere centred in the nucleus. Such cells could easily be mistaken for the neuroblastic cells, but they possess little affinity for either silver or gold stains (Text-fig. 2B and Fig. 6).

3. It is frequently found, even with ordinary aniline stains, that the undifferentiated cells, as described above, arrange themselves radially about areas of necrosis or vascular channels. They then assume the polar form, which is commonly described as that of the bipolar spongioblast. In general, such forms do not present the intensity of staining reaction to gold that occurs in the more organised cells of this form. However, it is possible that this appearance represents some differentiation of an early order.

4. At times bipolar forms of a more highly differentiated order occur about vascular channels. The processes are fairly thick and show a marked affinity for the gold stain. In appearance they are quite similar to the spongioblastic cells occurring in the ependymal tumours, and in one tumour occurring in a frontal lobe areas quite indistinguishable from the ependymoblastoma are present. It must be remarked that mitotic figures are scanty in this tumour, and it

is possible that it may be an anaplastic ependymoma occurring in an unusual situation (Text-figs. 2D and E).

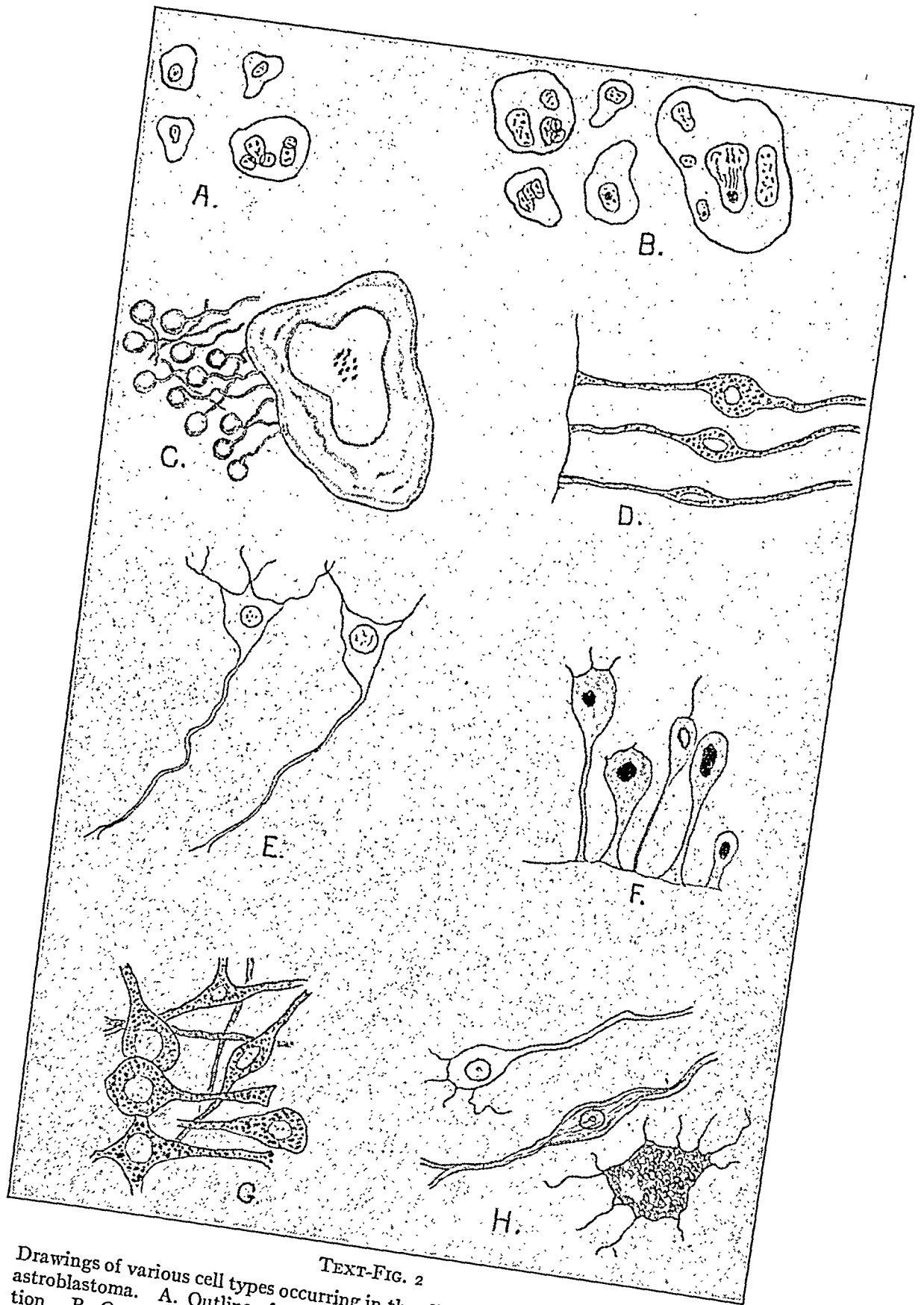
5. In five tumours astroblastic forms are prominent, and it becomes difficult to be certain under which category such a tumour should be placed. In three, however, the vascular response and frequency of mitotic figures indicate the glioblastoma.

The astroblast of normal histogenesis is a very typical cell. According to de Castro ³² a bipolar spongioblast secures a vascular attachment by one pole. This process hypertrophies and widens to a bell-shaped area at the point of attachment, while the other process atrophies. This formation marks the so-called astroblast. Fine processes emerge from the end distal to the blood vessel and thus gradually the adult astrocyte with its vascular attachment is formed. In the astroblastic stage glial fibrils have not differentiated.

Although the same type of cell is achieved in one of the above tumours the process of development is at times different. It commences as a columnar cell firmly set into the vessel wall, the nucleus being in that part of the cytoplasm distal to its attachment. The cell elongates, and by a constriction of the protoplasm near the vessel produces the first evidence of its vascular attachment and of its sucker foot. By a migration of the nucleus and its protoplasm still farther away, and with the formation of fine distal processes, the astroblastic form is assumed (Text-fig. 2F and Fig. 6). Such cells impregnate well with the metallic stains. Gold sublimate stains them brilliantly, but the silver carbonate methods show the cell form with more detail (Text-fig. 2E), while Cajal's pyridine silver is also effective in demonstrating them. They often present an extraordinary similarity to nerve cells and were, in fact, labelled provisionally by Greenfield ³³ as neuroblasts.

6. One tumour was found to be almost entirely composed of small cells, mainly of unipolar form. They stain selectively with the gold sublimate stain and present an appearance analogous to the unipolar spongioblast of histogenesis. They are much smaller than the bipolar or astroblastic forms previously described, but possess a fine vascular attachment (Text-fig. 2C and Fig. 7). The name spongioblastoma unipolare, as previously noted, would have been eminently suitable.

7. Certain of the tumours show numerous bipolar cells of an entirely different type from those already described. These cells will



TEXT-FIG. 2

Drawings of various cell types occurring in the glioblastoma multiforme and in the astroblastoma. A. Outline of anaplastic cells from a haematoxylin-eosin preparation. B. Outline of giant cells from the same tumour as Fig. 6. Haematoxylin-eosin. C. Small unipolar cells showing the relation of the processes to a thickened vessel, from the tumour shown in Fig. 7. Gold sublimate. D. Bipolar cells of the type seen in the ependymoblastoma. Gold sublimate. E. Outline of so-called astroblasts. Silver carbonate. F. The transition between a columnar cell set in a vessel wall and the astroblast. G. Various cells occurring in the astroblastoma shown in Fig. 10. Many of these cells resemble swollen protoplasmic astrocytes. H. Cells of the same tumour. Silver carbonate stain.

be described in more detail in the discussion of the polar spongioblastoma. The processes are long and frequently run in bundles, but the cells do not exhibit the special tendency exhibited by the other polar forms to radiate from or to be attached to vessels by these processes. On closer observation the polar expansions are frequently seen to break up into several strands. Many of these processes stain with Weigert's iron haematoxylin or Mallory's phosphotungstic acid haematoxylin stain, and the impression is gained that we are approaching an adult astrocyte cell of the polar, or of the piloid, type.

8. In certain tumours it was found difficult to separate the individual cells, despite the various staining methods employed. The appearance is that of a syncytium. These are all highly cellular gliomata, some with the vascular response of the glioblastomata, others resembling more a highly cellular astrocytoma. Bailey and Cushing regard the syncytial appearance so common in the glioblastoma as evidence of degeneration. In my experience this is not always so. In one example, which appears to be entirely of this nature, there is a remarkable preservation of neurones. The gold sublimate stain often impregnates this syncytium well. This appearance was common in gliomata in the region of the brain stem, although one occurred in association with a cyst on the basal ganglia of a child of 10 years.

Reynolds and Slater,^{34, 35, 36} in their studies of the gliomata, have paid some attention to this appearance. In a study of fifteen gliomata, they found thirteen fairly actively growing tumours. The edges of two appeared to be composed of a syncytium from which individual cells later separated. In three cases the gliomata were entirely syncytial, one having a history of over 4 years duration. They found all grades of differentiation between syncytium and astrocyte, but were uncertain whether the large astrocytes of the tumour could of themselves divide.

The syncytial appearance will frequently resolve itself in a remarkable way with the special stains. Thus, the small unipolar tumours described appear in paraffin sections to be entirely syncytial. There may also at times be observed in the astrocytomata syncytium-like areas, which, when impregnated with gold sublimate, are clearly not astrocytic, and these probably represent undifferentiated tumour cells. The syncytial tumours of a highly cellular

order, in my experience, contain fewer mitotic figures and a better preservation of glial and neuronc elements than the highly cellular glioblastoma.

Summary: 1. The glioblastoma is best considered as a locally malignant anaplastic tumour.

2. It may be associated with, and is possibly derived from, more adult tissue, for example, glia or ependyma.

3. Such cells may remain entirely anaplastic in character, or may assume in different degrees the more adult character of a spongioblastic, astroblastic or asteroid form.

4. At times the tumour cell may not de-differentiate to the apolar stage. The cells may be considered to multiply at an astroblastic, asteroid or polar stage.

5. The type composed of small unipolar forms is of different appearance from the ordinary type. This difference in form must not be interpreted too strictly in an embryogenetic sense. Chemico-physical agencies, such as pressure, oxygen starvation, and the presence of the products of necrosis, may have much to do with the shape and size assumed by the neoplastic cell.

6. It may be said that as a general rule tumours relatively rich in the more differentiated forms possess fewer mitotic figures than the less differentiated, but this rule is by no means absolute.

7. In some tumours the appearance of a syncytium is retained despite the use of special stains. Such a tumour is not of necessity degenerative.

ASTROBLASTOMA

This name is reserved for tumours in which so-called astroblasts are present in quantity. The characteristic appearance is that of numerous cells, each possessing one large single process of variable length which is either attached to a vessel wall or to a septum of connective tissue. The tumours are, on the one hand, related to the glioblastoma and, on the other, to tumours composed largely of protoplasmic astrocytes. They are tumours of the cerebral hemispheres of adult life, usually of diffuse type, and usually show degenerative changes. The average survival period of the Bailey and Cushing series of thirteen cases was 28 months. They are, therefore, slower in growth than the glioblastomata, but present some features in common.

Bailey and Bucy³⁷ have recently analysed twenty-five examples, besides giving an excellent general description of these tumours, and have also emphasised their inoperability.

The typical cell possesses a pyramidal body and a thick process attached to a vessel wall. From the other extremity of the cell fine processes may pass into the surrounding tissue, which frequently shows degenerative changes. These cells may be of asteroid or polar form and may show degenerative changes in the cytoplasm.

The origin of this type of cell has been discussed with the glioblastomata, where it often appears to be derived directly from a cuboidal cell, set in the vascular wall. However, such forms may arise by other means. Thus, degenerative changes occurring between the vessels lined by asteroid cells may result in a loss of all the processes except the vascular ones, and this appearance is not uncommon in the hypertrophied non-neoplastic astrocytes present at the advancing margin of a glioblastoma. Roussy and Oberling take this view of their origin and regard them, in fact, as being degenerated adult astrocytic forms. For this reason they decline to consider this tumour as a separate class, but rather as a degenerative type of astrocytoma. They note the appearance of similar cells in encephalitic lesions caused by radium.

It must also be noted that at times the large astrocytic forms present at the tumour edge may be introduced into a neoplasm as a vascular cuff, and produce a somewhat similar appearance (Fig. 8).

Tumours that fall within the category of astroblastoma are not all equally malignant. Some conform more to the glioblastic type, while others resemble the astrocytomata in their slow evolution. Thus, one of Cushing's cases gave a history of cerebral tumour existing over 15 years.

Three types of tumour have been described that might be included within this category:

1. Those examples that should probably be classified as spongioblastoma, of which four cases occurred in this series. A man, aged 29 years, developed signs of increased intracranial pressure and died within 4 months of the onset of symptoms. A small diffuse tumour was found deep within the left occipital lobe. Its general appearance was that of an undifferentiated, syncytium-like glioblastoma, but in certain situations these cells had, as previously described, formed definite astroblastomatous areas.

2. Type two is a very definite tumour type and corresponds to the appearance described by Bailey and Cushing. A woman, 47 years of age, presented signs of increased intracranial pressure. These rapidly progressed and death occurred after 5 weeks. A small, fairly well defined tumour was found at the junction of the right occipital and parietal lobes. Its edge was fairly clearly demarcated and its consistence somewhat greater than that of normal brain tissue. The tumour mass consists of areas of differentiated cells taking the gold stain intensely, set in massed form between dense connective tissue strands (Figs. 10 and 11). The main cell is large and pyriform, but with many bipolar and asteroid forms, while the cytoplasm of certain of these cells contains globular bodies that suggest degeneration. There is an entire absence of undifferentiated cells and mitotic figures cannot be observed. The cells border vascular areas, or connective tissue bands, and the tumour presents a general appearance of lobulation. The broad protoplasmic processes which pass from cell to vessel or stroma are often of considerable length in the deeply placed forms, and binucleated cells are not uncommon. Blood vessels are not numerous and degenerated areas are frequent in the central areas. The tumour cell, which is well shown by the gold stain, is clearly detailed by the silver carbonate method. The pyridine silver method results in impregnation of many of these cells, and one might well be excused for considering certain of them as neuroblasts. Fibrils can be seen in the cell protoplasm at times, but this is not a prominent feature. There is a considerable resemblance of these cells to those described by Roussy and Oberling in the *astrocytome giganto-cellulaire*.

3. Another type of tumour has been described as an astroblastoma, of which Carmichael quotes certain examples. These tumours seem, however, to be very closely allied to the astrocytoma and should possibly be included with them.

Summary: 1. The astroblastoma of Bailey and Cushing is a transitional type of tumour.

2. Cells of the appearance of astroblasts may be produced by a variety of means. Undifferentiated cells bordering vascular channels may pass directly into the astroblastic form without the intermediate spongioblastic form of histogenesis. Astrocytes may similarly, by a process of retrogression and possibly also by alteration in position, attain a similar appearance and arrangement about vessels:

3. The name astroblastoma should be confined to the type described by Bailey and Cushing. This may be regarded as somewhat intermediate between the astrocytoma and glioblastoma series.

4. In this series the majority of tumours composed mainly of so-called astroblastic cells showed numerous mitotic figures and were more closely allied to the glioblastoma. The two typical examples resembled more that type of tumour known as protoplasmic astrocytoma.

POLAR SPONGIOBLASTOMA

Bailey and Cushing examined examples of a tumour that they named a unipolar spongioblastoma. It was a firm, fairly well organised tumour of a much slower growth than the glioblastoma, the average survival period being 4 years. The site of election was the cerebellum of early years, but examples occurred elsewhere.

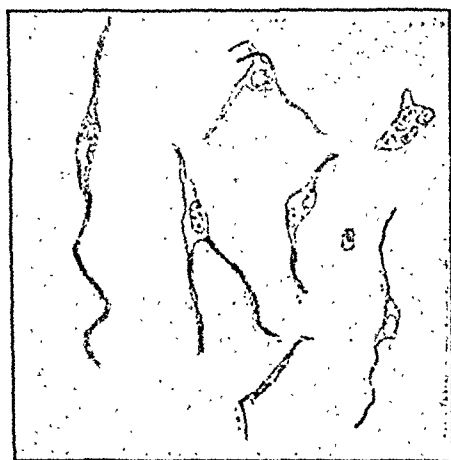
Microscopically, polar cells were found in an arrangement that suggested in low magnification an acoustic neurinoma. Between the cell bodies the tails ran in long, hard, wire-like processes, staining feebly with Mallory's phosphotungstic acid haematoxylin stain. Mitotic figures were rare.

That the so-called central neurinoma of Josephy is the same tumour is undoubted. The tumour is uncommon and the various authors who have encountered it have given almost every possible interpretation to the cell type. Bailey and Cushing¹ suggest that it may have attained this form through an attempt to differentiate along the lines of the neurilemmal sheath. Josephy⁷ likens it to the acoustic neurinoma. Roussy and Oberling reason along the lines of the neurilemmal sheath.⁸ As to the French school, the oligodendroglia is the representative of such cells in the central nervous system; they describe this tumour as an *oligodendrocytome fascicule*. Courville¹⁰ suspects that the polar cell is an immature nerve cell. Penfield has found no neuroglial fibrillae in this tumour and believes it to be a spongioblast.¹¹

It has been noted that, in the glioblastoma, areas of a somewhat similar appearance may occur. Penfield has remarked that this tumour is most often confused with the astrocytoma and must be distinguished from it by the absence of neuroglial fibrils. It has seemed to me that this type of tumour is midway between the glioblastoma multiforme and that type of astrocytoma in which fusiform or piloid cells are the prominent feature. Corresponding cells

occurring in the more organised type of glioblastoma multiforme have been noted previously.

A somewhat more organised type of tumour occurred in a child of 4 years, the survival period being only 4 months. The pons was largely replaced by a firm tumour, associated with a large central cyst. Microscopically, certain parts of this area present an appearance almost identical with that described by Bailey and Cushing. With the iron haematoxylin stain, in certain areas the dense stroma, in which the cells are set, shows many thick, well stained, wire-like processes (Fig. 12 and Text-fig. 3). Gold sublimate also shows the



TEXT-FIG. 3

Drawing of individual cells occurring in the tumour shown in Fig. 12.

cells clearly. Usually a large single fibril occurs in the tail of each cell. Other fibrils appear to be naked, but possibly possess a narrow protoplasmic sheath. However, other areas of this tumour show anaplastic forms, with many mitotic figures and giant cells, and are unquestionably highly malignant. In this tumour there is, therefore, a transition between what may be termed polar astrocytic forms, through polar forms without fibril formation, to ordinary anaplastic cells with giant cell formation.

A midline cerebellar tumour was examined in which polar cells without fibril formation are the prominent feature. There is no arrangement suggesting ependymoma and this tumour most closely preserves a pure spongioblastic form.

Another nodular tumour was examined in a woman 25 years of age. It apparently had arisen from the vermis and was growing into the mesencephalon. Although areas of this tumour are similar to

the description given of polar spongioblastoma, other areas are true astrocytoma of the fusiform or piloid type.

In a fourth tumour a firm nodular and lobulated mass had grown from the roof into the cavity of the fourth ventricle. The histological appearance is that of intersecting strands of fibres, and small and inconspicuous areas of polar cells (Fig. 13). This tumour is an undoubted astrocytoma of the subependymal type described by Roussy and Oberling.⁸ It is similar in structure to the small, multiple, subependymal granules occasionally encountered and is described here, as there seems to be a transition between tumours of this type and the glioblastoma multiforme with polar cell formation. To me the polar spongioblastoma seems to be a halfway house. The nature of the astrocytoma composed of polar forms will be discussed later, but I would raise the question whether it would not be better to omit this group. The more malignant forms fall into the glioblastomata, and the less malignant, without much stretch of the imagination, could be included with the astrocytoma. The typical cell may represent an attempt to form an astrocyte of piloid type, as described by Penfield. Such cells are not uncommon in subependymal tissue.

Summary: 1. Tumours in which polar forms predominate are described.

2. The polar spongioblastoma of Bailey and Cushing is possibly an intermediate form between a glioblastoma with polar cells and an astrocytoma with cells of a fusiform or piloid type.

EPENDYMOMA

Bailey and Cushing classed two types of tumours as arising from ependymal elements. In one group, consisting of five tumours, all of which arose from the floor of the fourth ventricle, the characteristic formation was an arrangement of well formed bipolar cells about thin-walled vascular channels.

The haematoxylin-eosin appearance was characteristic. Between a central vessel and a zone of nuclei lay a clear area, which consisted of those processes that passed from the cell body to the vessel wall. The gold chloride stain showed the individual cells in detail. These bipolar or ependymal spongioblasts are stained by such stains as Mallory's, which differentially stain neuroglial fibrillae; the same stain may show a collection of blepharoplasten in the cytoplasm

near the nucleus, although, according to Penfield, the importance of this point has been somewhat exaggerated. Mitotic figures were rarely found, the tumour being slower in evolution than the glioblastoma. It is named by Bailey and Cushing ependymoblastoma and is an extremely characteristic formation.

In the second type the cells were polygonal, but again showed blepharoplasten. Bailey and Cushing described seven cases, all being more organised tumours, but unfortunately not favourably situated for removal. One occurred in the cerebellar recess, three in the neighbourhood of the fourth ventricle, one arose from the septum lucidum, one in the region of the supramarginal gyrus and one in the occipital lobe. Fincher and Coon³⁸ studied eight cases, five of which occurred in the cerebral hemispheres.

These tumours may arise from ependyma in any situation, but also from areas where ependyma is not apparent. Small blind ependymal sacs have been shown to occur in the floor of the fourth ventricle, as well as in the central region of the spinal cord and in many other situations. The origin of these tumours from ependymal rests may be seriously considered, particularly when one observes the preponderance of such tumours in the spinal cord, where the possibility of the survival of ependymal remnants is obvious.

Kernohan, Woltman and Adson³⁹ found twenty-one ependymomas in fifty-one examples of intramedullary tumours of the spinal cord. They describe three definite histological pictures, all of which may be present in the one tumour:

1. In six cases canals lined by ependyma occurred. The cells were more columnar than normal, frequently showing blepharoplasten between the nucleus and the lumen.

2. In eight cases the characteristic appearance described under the term ependymoblastoma was present.

3. The third type is somewhat more controversial. It simulates the type of tumour described as neuro-epithelioma, which Penfield considers may be derived from ependyma,⁴⁰ and which Roussy and Oberling⁸ class as an ependymoglioma. Seven examples occurred, the characteristic arrangement being that of rings of cells, with scanty cytoplasm and small nuclei. They were frequently arranged about cavities containing mucinous material, which had almost the appearance of thyroid acini. Other parts of these tumours were, however, characteristic ependymoma.

The Roussy-Oberling classification is somewhat similar. They accept the two types described by Bailey and Cushing but rename the polygonal cell type *ependymocytome*.

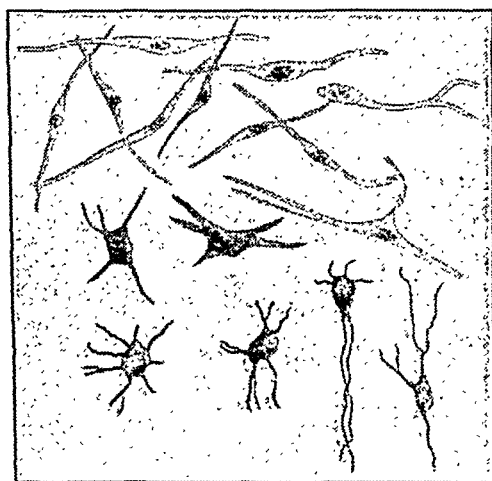
A third type is described as an *ependymogliome*, *ependymal cells* and *astrocytes* occurring together. A predominance of *astrocytes* may suggest an *astrocytoma*, but the presence of cavities lined by *ependymal cells* indicates the class to which this tumour belongs. In certain cases the *astrocytes* seem to be derived from the *ependymal elements*; in other cases the appearance suggests a proliferation of the two elements together.

The *ependymomas* were represented in the present series by five tumours, two of which occurred in one person. Four examples occurred in the midcerebellar region and one in the cerebrum. The cerebral example has been mentioned in discussing the *anaplasia* of more organised tumours. The cerebellar tumour that occurred coincidentally is extremely interesting from the standpoint of histology. Macroscopically, it shows two distinct formations of about equal size, one part being a fleshy, relatively avascular, lobulated formation, and the other showing vascular channels in abundance.

The first area resolves itself into *ependyma-lined lobes*, surrounding a core of tissue of a definitely *astrocytic* and *spongioblastic* nature (Figs. 2 and 14). The second area is typical *ependymoblastoma*, the vascular channels being surrounded by well formed *bipolar cells*. These cells are highly *protoplasmic*, with extremely well marked *protoplasmic processes* and large *vesicular nuclei*. At times the processes are observed to *bifurcate* at some little distance from the cell body. The vascular poles pass straight to the vessel walls, or run some little way alongside them before terminating. These typical so-called *ependymoblasts* are brilliantly impregnated by the gold sublimate method, and show transitional forms between the simple polar shapes and undoubted *astrocytes* (Text-fig. 4). In those areas where the vascular channels are less evident areas occur that might well pass for simple *astrocytoma*. Not only are *piloid astrocytes* present, but also clearly marked *asteroid forms* with *fibrillae*.

A second tumour, occurring in a woman 45 years of age, was situated in the cerebellum. In structure it is irregularly lobulated, well defined and easily shelled out from the cerebellar substance (Fig. 15). The microscopic appearance is regular and reveals the typical appearance of the *ependymoblastoma*, as described.

A third tumour is more remarkable. What appeared to be a simple cerebellar cyst filled with a horn-like coagulated content was sectioned in that area where the wall appeared to be thickest. The structure at first sight is not unlike the appearance of thyroid acini, the vesicles being filled with a homogeneous eosinophilic substance resembling that present in the central cavity. The walls are composed of a single layer of large epithelium-like cells, varying in form from a columnar to a somewhat flattened shape (Fig. 16). This thyroid-like appearance is very evident toward the central cavity



TEXT-FIG. 4

Drawing of polar and astrocytic forms within an ependymoblastoma, showing the apparent transition between these forms.

and the impression is gained that this was formed from a coalescence of these structures. Toward the cerebellar substance the tumour is composed of numerous polygonal or cuboidal cells, arranged less regularly, but forming here and there elementary acinar structures. No mitotic figures are present, the tumour appearing relatively inactive. There is no evidence of any spongioblastic formation, the cells being apparently true ependymal cells. The picture is remarkably similar to that frequently pictured as neuro-epithelioma, but as a similar picture is described as occurring in undoubted ependymomas, no doubt is felt that the tumour is of this nature.

Summary: 1. In reviewing these tumours we find two distinct cell types present:

(a) The first, representing adult ependyma, retains the adult shape and forms, or forms in places cavities and clefts.

(b) The second type, or ependymal spongioblast, is very characteristic. A modification of its structure results in a tumour astrocyte. Whether this cell arises from adult ependyma or from a subependymal element is not apparent. It is nearer in structure to the astrocytic series than to adult ependyma.

2. Nodular areas, if opposed to the ventricular surface, whether composed of astrocytic or polar forms, may be lined by adult ependyma. This does not of necessity imply that these latter cells are neoplastic.

3. As these tumours, whether composed of ependymomatous or ependymoblastic elements, are usually fairly well defined tumours without mitotic figures, the name ependymoma, as applied to them all, is for practical purposes sufficient.

OLIGODENDROGLIOMA

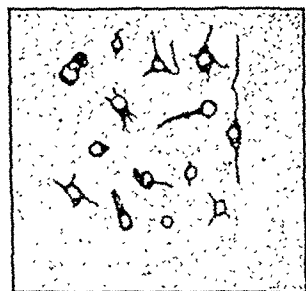
The prediction and proof that elements composed of oligodendroglia could of themselves enter into tumour formation is perhaps the crowning achievement of Bailey. For a while exact proof was wanting. Recently Bailey and Bucy⁴¹ have clearly demonstrated the oligodendroglial nature of the cells in these tumours and their work has been amply confirmed, among others by Kwan and Alpers⁴² and Roussy and Oberling.⁸

Such tumours may usually be identified by the characteristic appearance in haematoxylin and eosin sections. Masses of cells are found with small, round, hyperchromatic nuclei. The cytoplasm is poorly stained and each cell is enclosed by a rim of protoplasm with very little intervening stainable substance. They may occur singly or enclosed in groups by a denser stromal material producing a very characteristic boxed-in effect. This appearance is at times reminiscent of oligodendroglia, as seen in the stage of acute swelling. One cannot but be filled with admiration at the acumen of Bailey in deducing the nature of this tumour long before proof was obtainable.

With the Hortega silver carbonate stain, or one of its modifications, oligodendrocytes are observed. These cells are usually small and correspond to the small cortical oligodendrocyte of Robertson. The tumour is not, however, entirely composed of such cells. Many are quite unstained and may be of a much earlier form. This fact had led Penfield¹¹ to think that the most typical cell is in reality an oligodendroblast. Other cells are polar, while large types occur,

some of which appear to be giant oligodendroglia. Others, again, seem to be transition forms between oligodendroglia and classical neuroglia. Various transitions are described by Bailey and Bucy between oligodendroglia and frank astrocytes. The astrocytes observed by Kwan and Alpers were of a distribution that indicated many at least were inclusion forms. In the two examples encountered in this series any astrocytes observed were within the tumour edge and were undoubtedly inclusion forms, the reaction of neuroglia at the edge being very marked.

One tumour occurred in a man 22 years of age, who had presented for 5 months signs of increase of intracranial pressure. Death followed an attempt to localise the tumour by encephalography. A small nodule, about 2 cm. in diameter, was found abutting on a grossly dilated third ventricle in the region of the right foramen of Monro. Its origin was from the junction of the gyrus cinguli and the superior frontal gyrus. In haematoxylin and Van Gieson sections bands of collagenous material and small capillaries can be seen to alternate with areas of about equal width, containing a few small cells, often set in rows (Fig. 17). Their nuclei are small, round and fairly densely staining, while the cytoplasm is indefinite. The arrangement in rows suggests that of ordinary oligodendroglia and Martin⁴³ has described a tumour of similar appearance. In this tumour the characteristic boxed-in effect cannot be observed. Sections were impregnated by the Globus-Penfield modification of the Hortega method and by the modification recently suggested by Kwan and Alpers. In this particular tumour the former method gave the clearer impregnation. The majority of the cells are impregnated. The cytoplasm is variable in amount from a small ring of protoplasm, through uni- and bipolar forms, to well marked characteristic multipolar forms (Text-fig. 5). These bipolar forms are not interpreted as spongioblasts as they are not impregnated by the gold sublimate method and resemble the polar forms pictured by Hortega. No giant oligodendrocytes are present.



TEXT-FIG. 5

Drawing of oligodendrocytes from a similar area to that shown in Fig. 17. Penfield-Hortega stain.

A second example occurred in the left lateral ventricle of a man

26 years of age. The tumour had arisen in the roof of the anterior horn. The whole ependymal system showed innumerable small subependymal granules, the structure of which was astrocytic and the appearance not unlike that of the subependymal astrocytoma. In haematoxylin and eosin sections the main portion of the tumour shows the very characteristic boxed-in effect (Fig. 18), but other areas show the somewhat interfascicular appearance previously described in the last specimen. The blood vessels show the marked endothelial proliferation noted by Bailey and Bucy and others. With the silver carbonate stain the boxed-in areas are found to be composed of large swollen cells. The appearance is that of the typical swollen oligodendrocytes seen in the brain, thus confirming the conclusion of Kwan and Alpers that such areas represent degeneration. As, however, this appearance is rapidly assumed by oligodendroglia after death, it cannot be established that this appearance was of necessity present during life. Other areas show typical oligodendrocytes of the form previously described in Kwan and Alpers' four cases. Neither of my two examples shows calcification.

Roussy and Oberling describe two other tumour types that they consider to be composed of oligodendroglial elements. One is synonymous with the polar spongioblastomas, and the other they term a *gliome muqueuse*. This latter tumour elaborates a mucicarminophile substance of a similar nature to that observed in degenerating oligodendroglia. No examples that could be so interpreted occurred in this series.

Summary: 1. Two examples of oligodendroglioma were examined, each showing characteristic oligodendrocytes.

2. Calcification was not present in either tumour.

3. Endothelial hyperplasia was noted in the blood vessels of one tumour.

ASTROCYTOMA

This name is applied to certain superficially dissimilar tumours, the components of which are the adult astrocyte or some modification. The astrocytes in these tumours have been noted and clearly described for many years, and Stroebe's drawing,⁴⁴ published in 1895, presents an excellent picture of the various forms encountered in the ordinary type.

Bailey and Cushing subdivided the group into a protoplasmic

astrocytoma and a fibrillary type. Neither form is entirely pure and this subdivision, owing to their similar behaviour, has been discarded for practical purposes.

At the same time it is necessary to note the various forms that the tumour astrocyte may assume. The classical type, as usually described, is that of an asteroid cell, which may or may not have developed fibrillae. Penfield, whose knowledge of neurohistology merits our greatest respect, has found this type of cell to be the exception rather than the rule. Instead he finds the common cell to be piloid, that is, to be a somewhat fusiform cell from which fine fibrillary neuroglial processes emerge, to run in parallel strands. Such a cell is analogous to the piloid astrocytes occurring in the dorsal columns of tabetics. To Penfield, the protoplasmic tumour astrocyte is a degenerate cell of the type of the *gemästete Zell*, or plump astrocyte of Nissl, and its further evolution results in the giant astrocyte. Deprivation of oxygen will produce these bizarre forms in the brain.

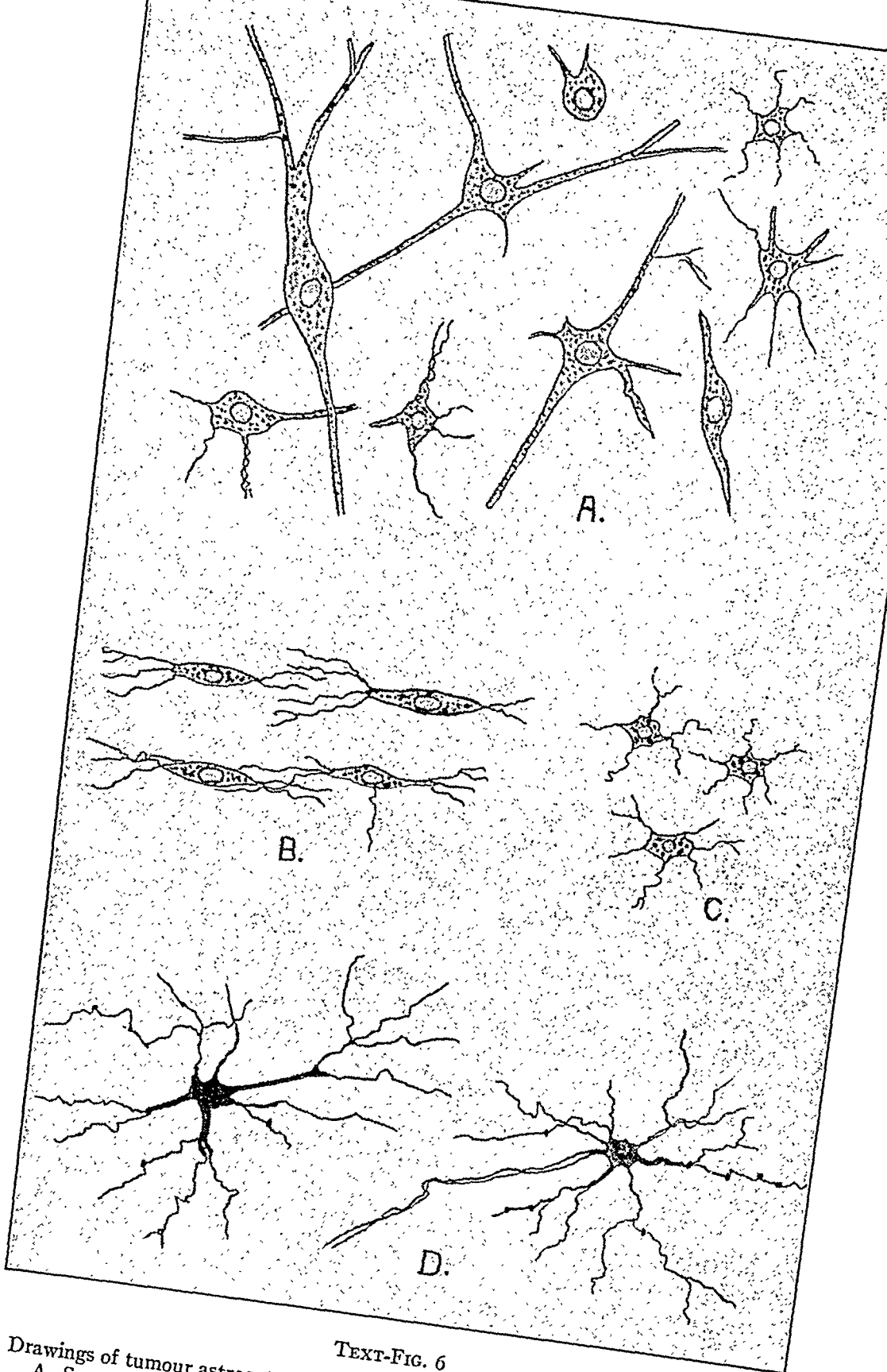
It is very difficult to be certain as to which particular influences produce the modifications of form occurring in tumour astrocytes. Such factors as the rate of subdivision of the tumour cell, oxygen starvation, and the presence of degeneration products must all modify the form assumed. The cell shape is again modified by the position in which the cell grows. In Text-figure 6B the fusiform-shaped astrocytes occurred in the region of a superior cerebellar peduncle, where their position between nervous strands determined the piloid form. Nearby in the region of the substantia gelatinosa the asteroid form was assumed (Text-fig. 6C).

Again (Text-fig. 6D), typical fibrous astrocytes were present in that part of an astrocytoma in the region of the *iter* where there was little obstruction to the full growth of the star-like expansions.

There is considerable variety also in the form assumed by the tumour. On the one hand is found a circumscribed nodule, often associated with a cyst, as in the cerebellar type so admirably described by Cushing.¹⁶ On the other, a diffuse type occurs which may be intimately mixed with more or less normal brain tissue. This tumour, if very cellular, may be of the type of the diffuse protoplasmic astrocytoma, but if the cells are sparse and fibrilla formation is marked there may be little to distinguish it from diffuse glioses of the type of syringo-myelia, or from certain forms of cerebral sclerosis.

The so-called protoplasmic astrocytoma is composed of cells in which the protoplasmic body and its processes are very prominent, and in which few neuroglial fibrillae are present (Text-fig. 6A and Fig. 19). Tumours largely constituted of such cells may occur in both cerebellar and cerebral hemispheres. Cushing¹⁶ has lately withdrawn many of his protoplasmic astrocytomas of the cerebrum. He notes that "many of the latter are rapidly growing tumours, with abundant mitoses, and should in all probability be separately classified. They, moreover, occur in adults with the average age of forty years." This note is significant. In the early description by Bailey and Cushing the view was expressed that the spongioblastoma multiforme might be derived from the cerebral protoplasmic astrocytoma. It is probable then that many of these latter might be better classified with the anaplastic glioblastomata. A transition of this type occurred in this series.

A man of 43 years, suffering from headache and vomiting, with mental obtundity, was found to have well marked signs of increased intracranial pressure. Owing to the presence of a motor paresis of the left side of the body, the brain was explored on the right side. A large, diffuse, inoperable tumour was found involving a considerable part of the parietal cortex. Although a small superficial biopsy portion showed many astrocytes the diagnosis of astrocytoma was not definitely made at this stage, owing to the well known astrocytic reaction that may occur at the edge of various tumours. A large cerebral hernia developed and the patient, although temporarily improved, died a few months later. At autopsy an enormous tumour was found involving the right parietofrontal area and limited on the inferior surface by the roof of the lateral ventricle. The growing edge, which was well anterior to the area adjacent to the cerebral hernia, is typical astrocytoma, and shows a multiplicity of protoplasmic forms without mitotic figures (Text-fig. 7 and Fig. 20). The deeper parts adjacent to the lines of tension induced by the protrusion of the tumour into the area of hernia are grossly cellular and present the typical appearance of the glioblastoma (Fig. 21). Mitoses, giant cells and vascular changes are in abundance. The majority of cells are typical of anaplastic glial cells and are not stained by the specific methods. Among them are irregular forms staining deeply with the gold sublimate, and without doubt of a similar nature to those occurring in the less active edge of the

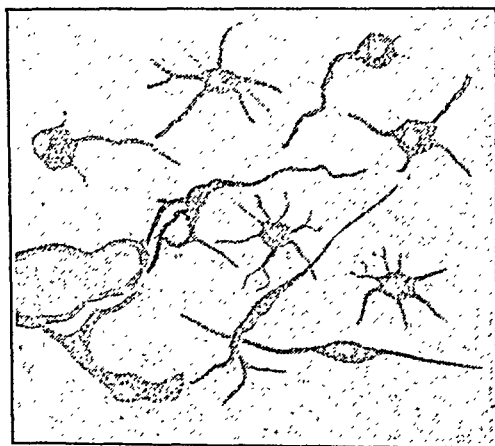


TEXT-FIG. 6

Drawings of tumour astrocytes as seen in various astrocytomata.
 A. So-called protoplasmic astrocytes.
 B, C. Asteroide and piloid forms occurring in different situations in the one tumour.
 D. Fibrillary astrocytes.

tumour. It is not possible to be certain that this glioblastic transformation occurred subsequent to the change in tension induced in the tumour by the decompression operation. It is quite possible that invasion of the ventricular wall may have produced this change. At the same time no doubt could be entertained but that the central part of an astrocytoma had become more anaplastic and produced the typical picture of the glioblastoma multiforme.

Consideration of the forms of tumour cells in these so-called protoplasmic astrocytomata leaves the strong impression that the so-called astroblasts and spongioblasts result from haphazard varia-



TEXT-FIG. 7

The variation in shape of the astrocytes in a protoplasmic astrocytoma of the cerebrum.

tions in the shape of cells at a more or less similar stage of maturity. The same embryonic forms seem to the author often to represent stages of decay rather than the stages of histogenesis. The appearance often suggests that such cells divide amitotically as astrocytes, or as a more amoeba-like or undifferentiated cell.

In their atlas, Roussy and Oberling⁸ describe the varying histological pictures of the astrocytomata. They are not hampered by any desire to force their tumours into a classification based on embryogenesis, but accept the subdivision into a protoplasmic and into a fibrillary group. The variations that they describe are based on morphology and are extremely instructive.

One type of fibrillary astrocytoma is the *gliome sous-épendymaire*. The characteristic cell is fusiform with processes emerging from the poles. The contained neuroglial fibrillae are very thick and their intracellular origin appears with particular clarity. Such tumours

may arise from the fusiform neuroglial elements, which reinforce the ependymal lining of the ventricular system. The small, multiple, subependymal granules, which are at times seen, present a very similar appearance. The relation of cells of this form to the polar spongioblastoma was discussed previously and a typical subependymal astrocytoma was mentioned.

The *astrocytome giganto-cellulaire* is another characteristic form and conforms to the amoeboid glioma of Lotmar.⁴⁵ The body of the cell is bulky and the shape variable (Fig. 22). The cytoplasm often shows appearances suggesting degeneration, and contains granules and lipid droplets. The nuclei are often multiple; they may be pyknotic and peripherally placed. The processes may be long and thick, and occasionally neuroglial fibrillae are present. The likeness of these cells to ganglion cells is at times striking. Two tumours of this nature occurred in this series, each being circumscribed and well defined. One occurred in the midline of the cerebellum and the other in the frontal lobe, each being associated with a well defined cyst. In the small cerebellar nodule few neuroglial fibrillae are present, but they are numerous in certain areas of the example occurring in the frontal lobe.

Similar cells occur in the astroblastoma and Roussy and Oberling see in this a degenerate astrocytoma.

The *astrocytome pseudo-papillaire* of Crouzon and Oberling⁴⁶ presents an appearance very similar to the Bailey-Cushing ependymoblastoma. As certain of the examples develop in the neighbourhood of the ventricular system and project into it, it is possible that this type is identical. That type occurring away from the ventricular system is considered by Roussy and Oberling to consist of astrocytes set in a similar radial fashion about vascular areas, but Bailey and Bucy³⁷ consider the example demonstrated by Crouzon and Oberling to be an astroblastoma. The close relation of the so-called ependymoblasts and astrocytes has been discussed under the section dealing with ependymomata. The reconsideration of similar forms from a somewhat different angle in this section illustrates again the essential unity of ependyma, subependymal elements and asteroid glia.

Summary: 1. A description of the cell forms encountered in tumours of adult glial origin is given. Many of the cell shapes that have been described with names of an embryogenetic significance appear to the author to be modifications of the one adult stage.

2. Polar and piloid forms may arise in subependymal areas. They may also arise from the position of an astrocyte, which prevents the development of its processes in other than a certain plane.

3. Other factors, of which one may be anoxaemia, result in a considerable increase in the bulk of the cytoplasm. Such cells are usually known as protoplasmic astrocytes. In more advanced states of swelling the cytoplasm may be extremely bulky and show marked evidence of degeneration.

4. So-called astroblasts may occur as a result of degeneration in mature cells, by which certain of their processes may be lost, while those processes attaching them to vascular channels, and which are presumably in an area more favourable for nutrition, are unaltered, or even hypertrophied.

PINEALOMA

For many years tumours arising from, or in the vicinity of, the pineal body have been described under a variety of names. Krabbe⁴⁷ was the first to recognise their real origin, and to describe them as pinealomata. Bailey and Cushing subdivided them into pineoblastomata and pinealomata. The pineoblastoma, through a consensus of opinion, has now for practical purposes become merged with the medulloblastoma. The pinealoma persists as a definite type.

Some knowledge of the development of the pineal body is necessary to understand the varying histological pictures that occur in tumours of this type. This cannot be fully entered into here but is well discussed by Globus and Silbert.⁴⁸ Two types of cell are usually observed, a large cell with a clear vesicular nucleus and much cytoplasm, which may contain mitochondria, and a small cell with scanty cytoplasm and a deeply stained nucleus. The former cell is of a parenchymatous type. It appears to develop from the medullary epithelium, as do the various glial and ependymal forms, and may have some glandular function at an early stage. The small cell seems to be mesoblastic in origin, and may be transformed into ordinary connective tissue.

These facts are clear and well recognised. There is, however, a difference of opinion about certain features. Horrax and Bailey⁴⁹ believe that glioblastic elements persist in these tumours and that a spongioblastic pinealoma may occur. In addition, they have re-

cently described a pineal tumour containing typical nerve cells which they believe to be neoplastic. Schmincke⁵⁰ has also described what he believes to be a true ganglioneuroma of this region. Globus and Silbert, on the other hand, believe that such elements are not represented in the normal pineal body, and when found in a pinealoma are of the nature of inclusions.

The pinealoma presents a variety of pictures. It may show a formation corresponding to the primitive mosaic, with the large clear cells outlined by the small dark cells, or both cell types may be intimately mixed. Various degrees of transformation may also be noted between these small cells and the connective tissue stroma. If this transformation is marked the appearance of the adult pineal body is approached, with the alveolar pattern of the large cells enclosed in connective tissue stroma. In addition, multinucleated giant cells are of common occurrence. Globus and Silbert consider these to be of a foreign body nature and to be most marked in those areas where regression is occurring.

Tumours in the position of the pineal body occurred in two cases in this series. One occurred in a child. A tumour of the size of a large walnut was found lying upon and invading the quadrigeminal plate. The pineal body was not present. The tumour cell is large, with abundant cytoplasm and a large vesicular nucleus (Fig. 23). It differs markedly, however, from the usual cell of the pinealoma in that mitotic figures are extremely numerous. In addition, although the cerebellum was not invaded, the tumour was in active growth in the subarachnoid spaces between the folia. Multinucleated giant cells are frequent and are undoubtedly of neoplastic origin. The nuclei are at times peripherally placed. Usually the cell centre is, however, crowded with a knot of nuclear forms or is occupied by an enormous, poorly stained nuclear mass. Although such forms indicate insufficient nutrition they are not confined to the more degenerate areas. They are, however, larger and more abundant in regions where the wide spacing of the cell units suggests an oedematous condition. None of the typical small cells is observed. Blood vessels are extremely numerous but thin-walled.

Hypertrophic astrocytes are present at the invaded margin, but are absent from the tumour proper. In the same way degenerating nerve cells and their processes are present in that part of the tumour which has invaded the quadrigeminal plate, but in no other portion.

Connective tissue strands are present throughout either in prominent strands or more intimately mixed with the tumour cells.

In the other example the tumour was grossly necrotic and no definite histological picture could be obtained.

Summary: 1. In one example of pinealoma only the large parenchymal cells were present.

2. The tumour was unusual in that it was highly malignant and invading the subarachnoid space.

3. No astrocytic or neuronc forms were observed within the true tumour zone, although present at the edge.

GANGLIONEUROMA

Tumours composed of nervous elements present considerable difficulty. They fall into two main groups, one composed in the main of adult nerve cells, and the other of forms that suggest neuroblasts. The former are among the rarest of tumours, none occurring in the Bailey-Cushing series, but the latter exist in considerable numbers.

Well differentiated cells may be identified by the nucleus with a prominent nucleolus, and by the presence of Nissl bodies and neurofibrils in the cytoplasm and in the processes. This having been done, it must be proved that these are true tumour cells and not preserved nerve cells within a tumour resulting from invasion of a heterotopic or of a normal nuclear mass. There must be such evidence of division of nerve cells as binucleated forms, or proof that the adult forms are derived from subdividing, less differentiated forms. If these criteria are accepted the number of well differentiated ganglioneuromata occurring within the cerebrum will be exceedingly small. The cerebellar type differs somewhat from the cerebral type, being much more diffuse, and has been well described by Bielschowsky and Simons.⁵¹

A considerable number of examples of ganglioneuromata, or gangliomata, have been reported in which the cells are not so evidently true ganglion cells (Fig. 24). Courville has made an extensive survey of the entire group and has summarised the majority of examples of both types. Other examples have since been reported.^{52, 53, 54} It is probably better at present to preserve an open mind concerning the true nature of many of these tumours. Tumour astrocytes may be exceedingly bewildering, not only in their general

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shape but in the appearance of the nucleus. One may take individual cells from their surroundings and be quite unable to say on morphological grounds whether such a cell is a neuroblast or an astrocyte. True neurofibrils and Nissl bodies are usually absent from these cells, and the appearance of the nucleus may be extremely deceptive. Cells showing a nucleus with a large central nucleolus must not be considered as neuroblastic, unless there is undoubted evidence of the presence of neurofibrils and Nissl bodies (Figs. 25, 26, 27). Many of the large protoplasmic tumour astrocytes may also be impregnated with certain of the silver stains; and, if the cytoplasm contain granular degeneration products, they may suggest by their appearance the presence of Nissl bodies.

PART II

THE PRESERVATION OF NEURONIC ELEMENTS

It is frequently difficult in any neoplasm to be sure as to which is tumour cell and which the cell derived from the invaded tissue. The stroma is a reactive structure, which, although it supports the vascular supply of the tumour tissue, often presents an appearance that suggests an attempt to confine and isolate the neoplasm. This particular reaction in ordinary body tissues is a reaction of the supporting and vascular tissues and is exhibited by elements of mesoblastic origin. Thus, when a mesoblastic tumour invades a tissue of the same origin it may be difficult to say whether a particular cell is neoplastic or not.

At the same time certain elements invaded by a tumour, even if highly specialised in function and of non-mesoblastic origin, may show a singular degree of resistance. Thus the small bile ducts may remain isolated in a carcinoma invading the liver, or a peripheral nerve may survive in the midst of a carcinoma nodule. Even fine, non-medullated nerves may persist in ordinary body carcinoma and attempts have been made to prove that the appearances of these represent regeneration and innervation of the tumour mass.⁵⁵

It is often a very striking feature of the silver stains, as applied to glial tumours, that numerous nerve fibres seem to pass through the neoplasm, and present no great evidence of degeneration. The small degree of loss of function in comparison with the actual zone of invasion is certainly related to the survival of these elements. The

incidence of sudden vascular changes, however, produces an immediate interruption of function and is responsible for much of the symptomatology of infiltrative tumours, which may amaze the pathologist by their size.

Not only may nerve fibres be observed deep within these neoplasms, but at times, and in particular in the vicinity of vessels, the arrangement of non-medullated fibres may suggest actual regeneration.

Cajal,⁵⁶ in his classical work, has reviewed the subject of neural regeneration within the central nervous system. By numerous experiments he has shown that regeneration is attempted, but is grossly inefficient. It is usually confined to neurofibrillar activity within the proximal part of an injured axone, and in occasional feeble and abortive sprouts. The experiments of Tello⁵⁷ suggest that this poverty of response is not so much the property of these particular neurones, as the absence of some substance with a neurotropic effect. He has grafted within the cerebrum segments of degenerating peripheral nerve containing bundles of multiplying neurilemmal cells. An abundant outgrowth of neurofibrillae was produced from neighbouring divided nerve tracts in the direction of the graft. It is presumed that it is the absence of true neurilemma or some kindred element within the central nervous system that determines the poverty of regeneration.

Bielschowsky⁵⁸ in 1906 found at the margin of and within gliomata appearances that were identical with the phenomena of regeneration, as observed within peripheral nerves. These took the form of fine non-medullated nerve fibres, at times arranged in perivascular plexuses. The axones followed atypical paths and frequently terminated in *cônes de croissance*, in rings, or in more complicated fenestrated structures, all of which may be observed in regeneration. Herxheimer and Gierlich,⁵⁹ and Miyake,⁶⁰ separately observed similar appearances at a later date, but concluded that the fibres represented the remnants of preëxistent nerve fibres, rather than regeneration. Cajal, however, accepts certain evidence as showing the possibility of regeneration.

Thus senile plaques have been observed by Fischer⁶¹ to show undoubted evidence of penetration by neurofibrillae. Newly formed dendrites have been noted in the pyramidal cells in tuberosc sclerosis (Bielschowsky and Gallus⁶²), and in the pyramidal cells in

Ammon's horn in senile dogs (Lafora ⁶³). Spatz,⁶⁴ too, has noted similar appearances in the so-called myelin spots.

It may be said then that the occurrence of newly formed neurofibrillae is not an impossibility in the presence of some unknown neurotropic substance, which may be present in degenerating areas. In a severe injury to an axone in the central nervous system, however, the distal part completely degenerates. The proximal part forms a retraction ball, as shown by Cajal, with at times some feeble attempts at regeneration. Finally the area atrophies to the level of the nearest collateral, which usually hypertrophies.

The nerve cell, if severely injured, rarely recovers, the evidence for the subdivision of nerve cells within the central nervous system being extremely scanty. Many so-called appearances of subdivision have been subdividing neuroglial cells, although binucleated pyramidal cells have occasionally been observed.

The presence of nerve fibres within the gliomata, although denied by Virchow, has been known since the end of the last century. The Weigert stain for the medullary sheaths does not show the true extent of their penetration. Many of the nerve fibres, on entering certain of the gliomata, may lose their myelin sheath and persist as naked axones. These latter can be satisfactorily demonstrated only by the silver methods.

The presence of nerve fibres was noted in varying amounts in the majority of the various gliomata examined in this series. In the astrocytomata they were fairly constantly present.

The photograph (Fig. 28) is from an area of a diffuse astrocytoma well within the edge. As the tumour extended from the vertex to the middle of the left frontal lobe and was of a slowly growing type, it is obvious that it must have been present for many years. The first ascertainable symptoms occurred only 6 months before death, which followed operation. They then were due to a general increase in intracranial pressure rather than to any focal lesion. This gives us undoubted proof that the enclosed neurones were not notably diseased, but in fair physiological health. It is probable that the absence of marked haemorrhagic or necrotic changes within tumours of this type explains the preservation of the nerve elements. The neoplastic astrocytes are not of themselves markedly destructive of the neurones, which are readily preserved until too gross a gliosis occurs. They may then degenerate owing to the effects of pressure,

or owing to the necrotic changes engendered by interference with their blood supply.

The glioblastoma differs considerably from the astrocytoma. Haemorrhage, thrombosis and degeneration produce marked hiatuses in the invaded network of axones and their collaterals. The result is that it is in the neighbourhood of the vessels that the persisting nerve fibres are mostly found. Appearances suggesting a perivascular plexus are often very striking and one of the original examples described by Bielschowsky,⁵⁸ judging by the illustrations, was undoubtedly an example of the glioblastoma multiforme.

These so-called plexuses probably occur owing to the ingrowth of new vessels through a network of nerve fibres; their early appearance may be observed in the areas that are becoming vascularised beyond the tumour edge.

In non-degenerative areas of the glioblastoma a persisting network of naked nerve fibres is not infrequent. The impression is formed that it is less the neoplastic cell than the effects of degeneration and haemorrhage that produce the damage to these elements. Secondary carcinoma forms a strong contrast in this regard. Although a certain number of axones may persist and even present appearances similar to those described by Oertel⁵⁵ in the innervation of tumours elsewhere, they mostly degenerate speedily.

In one example of glioblastoma the persistence of neuronie elements is notable. This tumour is less degenerative than is usual, but is highly cellular. It is composed of undifferentiated cells set in a syncytium-like arrangement, but showing the typical vascular hyperplasia. It extends from the temporal lobe through the thalamus and hypothalamic regions into the upper part of the pons, and has involved much grey matter. Considering its extent, the paucity of symptoms was extraordinary. In all areas preserved nerve cells with their processes are abundant (Fig. 29). So marked is this feature that sections of certain areas superficially resemble those of the adult ganglioneuroma. The nerve cells are not entirely healthy, but present evidence of degeneration and marked alteration of the neurofibrillar content, although these appearances may be the result of postmortem changes.

Figures 30 and 31 are from a tumour removed at operation from a child's cerebellum. On account of a tangle of nerve fibres and cells, in conjunction with undifferentiated elements, it had been classified

as the rare adult ganglioneuroma. There does not seem to be any evidence of intermediate forms between the two types, and in addition the nerve cells seem to be mostly degenerative. It is considered, therefore, that such cells are inclusion forms, or represent a heterotopic rather than a tumour formation.

It is usual to find nerve fibres, and even occasional nerve cells, within the medulloblastomas. In one example the penetration of unaltered axones is marked (Fig. 32). Another extremely malignant example shows persisting nerve cells well within its substance, which are apparently derived from a strand of almost unaltered nerve tissue in the vicinity (Fig. 33). In Figure 52 of the Bailey-Cushing monograph, a multipolar neuroblast is pictured in the substance of a medulloblastoma. This appearance is strikingly reminiscent of a nerve cell inclusion, as are also the neuroblasts shown in Figures 50 and 51. Cajal noted that in animal experimentation degenerating nerve cells of the smaller type may assume the appearance of neuroblasts. It must be shown clearly that an area said to contain neuroblasts has not involved any nuclear mass. Even so, the question of the identification of neuroblasts is difficult, as has already been pointed out.

Cajal has also pointed out the phenomenon of preserved nerve fibres. In experimental lesions neurones may be preserved from the effects of autolysis by exudate and, although dead, present the appearances of exquisite normality with a clear, fine definition of nucleus and neurofibrils. Thus an instantaneous death may produce the appearance of unaltered, live neurones, an appearance that has also been noted by Marinesco⁶⁵ in vascular lesions.

In the tumours examined no appearances were noted that would offer unequivocal proof of regenerative attempts on the part of the neurones. Fine networks may be noted about blood vessels, but *cônes de croissance* and similar appearances are rare. Occasionally a somewhat fenestrated net is observed, but it is extremely easy to read into such appearances deductions that are not correct.

Summary: 1. Intact neurones are frequent in the gliomata, particularly in the more slowly growing, non-degenerating types.

2. In the degenerating parts of rapidly growing tumours naked nerve fibrils may persist about vascular channels. Networks and plexuses were observed, but did not present appearances suggesting regeneration.

3. Included nerve cells may mimic neuroblasts. The difficulties in distinguishing neuroblasts from other neoplastic cells are discussed.

THE MESOBLASTIC CONTENT

The reaction of the fibroblastic and vascular structures will not be considered. It has been amply described by many authors. The differentiation of mesoblastic derivatives from glial tissue usually presents little difficulty, although certain authors have described a transition between the two.⁶⁶ The reaction of the cellular lining of the Virchow-Robin space has also been described, and is according to Bailey and Bucy of some importance in the astroblastomata.

Microglia has formed the subject of many papers. Penfield⁶⁷ has admirably described its reaction about the gliomata and shown all stages of the transition between it and the compound granular corpuscles.

THE OCCURRENCE OF OLIGODENDROGLIA

Oligodendroglia, although of the same origin as neuroglia, will be discussed only shortly here. Owing to its fragility it is not usually well stained about tumours in postmortem material. According to Roussy, Lhermitte and Oberling,⁶⁸ and lately Cramer and Alpers,⁶⁹ it too may give origin to compound granular corpuscles.

At the same time perineural satellitosis has been observed by Bailey and Bucy about the oligodendrogliomata.⁴¹ Otherwise its activities appear to be inconspicuous.

THE PRESERVATION OF CLASSICAL NEUROGLIA AND ITS REACTION FORMS

A full understanding of the reaction of ordinary classical neuroglia, fibrous and protoplasmic, is of great importance if the histology of the gliomata is to be fully comprehended.

The glioma is a neoplasm of glial origin, invading glia. As classical neuroglia is a labile tissue analogous to ordinary connective tissue, it is only to be expected that it should react to an irritant or foreign body as does connective tissue. This is indeed so in the brain, but the reaction of neuroglia is slow as compared with its analogue in ordinary body tissues. Even so, it may very effectively encapsule an irritant body, provided that body is not too noxious and gives it the necessary time for proliferation and the production of fibrils.

The neuroglia of the grey matter is known as protoplasmic neuroglia. This differs from the fibrous neuroglia in the white matter in having a higher cytoplasmic content, branching protoplasmic processes, and a structure free from fibrils. Protoplasmic neuroglia may, however, produce fibrils and become typical fibrous neuroglia. As the white matter is approached from the grey, transition forms appear. Both types show the usual thick processes of attachment to neighbouring vessels.

The older methods of staining, such as those of Weigert, although showing the fibrils well, did not demonstrate clearly their relation to the cell cytoplasm. They were considered then to be structures independent of the cell body. The modern methods of staining, as the Cajal gold sublimate and the Hortega silver methods, have emphasised their close relation to the cell. It is now held in many quarters that glial fibrils are always continuous with the glial cell, and have in fact a narrow protoplasmic sheath. It would, however, be difficult to believe that they are dependent for their perpetuity on the cell, as in a dense gliosis cells may hardly be evident. It is probable that the fibrils may survive even when the cell is defunct.

It is widely recognised that in the presence of cerebral neoplasms glial cells may hypertrophy and proliferate, resulting in an increased number of large glial forms. This reaction is dependent on the nature of the irritant. A rapidly growing neoplasm, provided it is not too rapid and too noxious, will produce many hypertrophic forms, while a slowly growing type may produce few. However, even in these examples, although actual cells may be scanty, there may be a marked increase in the glial fibrils at the border of the tumour. Thus a slowly forming cyst, or a compressing meningioma, may produce a dense reactive gliosis. A similar gliosis may occur subsequent to the degeneration of nerve cells and tracts, whether the process be inflammatory, toxic, or simply abiotrophic.

Apart from this gliosis, increased numbers of neuroglial cells and hypertrophic forms have for many years been noted at the margins of tumours. They occur at the invading margins of certain secondary carcinomata, sarcomata, and even about the transplanted sarcomata of rodents, as well as about the gliomata.

The study of the margins of secondary carcinomata in the brain forms an excellent comparative study, for here there can be no question as to which is glial reactive cell and which is neoplastic. In some

examples there is a marked glial reaction, in others the zone of degenerative material about the tumour is so great that these glial forms are hardly evident.

The study of one small nodule of adenocarcinoma proved instructive. It is 5 mm. in breadth and so situated in the cerebral cortex as to be half within the grey matter and half within the white. About that area which borders on the grey matter, a dense but narrow zone of enormously hypertrophied protoplasmic astrocytes occurs. In the area adjacent to the white matter there is no reaction, and the astrocytic band ceases accurately on each side at the junction of grey and white matter.

Other examples of secondary carcinoma so far studied serve to show, although less dramatically, that the grey matter is more productive of these reaction forms than the white. Away from the margin of the neoplasm fairly normal protoplasmic astrocytes may be seen amidst degenerating nerve cells. By a fairly rapid transition these forms have become huge at the tumour margin. The cell body and thick processes are densely stained with the gold sublimate. Their vascular attachments are prominent, heavy and often multiple. Not only do the long processes pass to the vessels, but into the interstices of the adenomatous nodules, and occasionally a process may appear to be attached to the neoplastic wall. Compressed astrocytes may at times seem to be contained within the interstitial tumour tissue, but this is unusual. It is interesting to speculate on the capacity for alteration in position these protoplasmic astrocytes may possess. Roussy considers, contrary to general opinion, that classical neuroglia of both types may produce the amoeboid and phagocytic compound granular corpuscles.

Cajal⁵⁷ believes in the migratory capacity of astrocytes. In experimental wounds he finds that if a small vessel be coagulated, the sucker foot and vascular processes of an attached astrocyte will become poorly stained and even undergo granular disintegration preparatory to migration. In other necrotic or thrombotic areas he has observed a migratory flight of astrocytes away from the presumed noxious or poorly oxygenated area. "The pedicles of the neuroglial cells all orientate themselves in a direction opposite to the loss of substance. These appendices are parallel and two to three times their normal length." They are stretched out in the direction of the line of flight and the appearance in their figure is strongly reminis-

cent of the streamers of astrocytic processes coming from between the masses of the advancing adenocarcinoma. He regards neuroglia as having this amoeboid capacity of losing the unsuitable attachment, of migrating and finding fresh vascular attachments elsewhere. Certainly the appearance at the edge of carcinoma nodules, particularly if their small alveolar structure is preserved, might suggest this, by the heaping up of the hypertrophied astrocytic forms and the passage of long thick processes in parallel between the nodules of the advancing neoplasm.

As noted, the white matter is much less prodigal of its astrocytic forms. Hypertrophic types, however, occur and may be fairly prominent. Possibly owing to the fact that they are placed between parallel nerve tracts they are not so apt to assume the asteroid but more a piloid form, which is particularly readily seen if the section is cut in the direction of the longitudinal tracts.

At times the glial proliferation about secondary carcinoma assumes such proportions as to become a problem of practical importance. Carmichael¹⁸ has noted about a carcinoma nodule a gliosis that was so marked as to resemble the appearance of astrocytoma. As the nodule required some little search to find it is apparent that again we have a demonstration of the pitfalls awaiting those who deduce too much from the examination of small pieces of tissue alone. Certain of Cushing's¹⁶ earlier reported cerebellar astrocytomata have proved, with further study, to be a reactive gliosis about non-glial tumours. The angioblastomata in particular were confused by this deceptive gliosis.

Such a study affords a basis of contrast and comparison for the study of similar reaction forms at the margins of gliomas. The more rapidly spreading types are frequently productive of much astrocytic proliferation; the more slowly growing types as a rule produce less, although there may be much fibrillar gliosis.

One considerable difference, however, between these tumours and the carcinoma group is observed. In the latter form any contained neuroglial forms are speedily destroyed. In the gliomata they may find an environment somewhat of their own kind, and may not only remain embedded in the mass, but proliferate and produce many unusual forms. The great enemy of these glial cells is less the tumour cell than the effects of vascular thrombosis, haemorrhage and cell necrosis. If the tumour is not of the degenerative type such forms

may persist in quantity, varying from fairly normal forms in some of the slowly growing tumours to hypertrophic forms occurring in those tumours where a hypertrophic reaction is found at the edge.

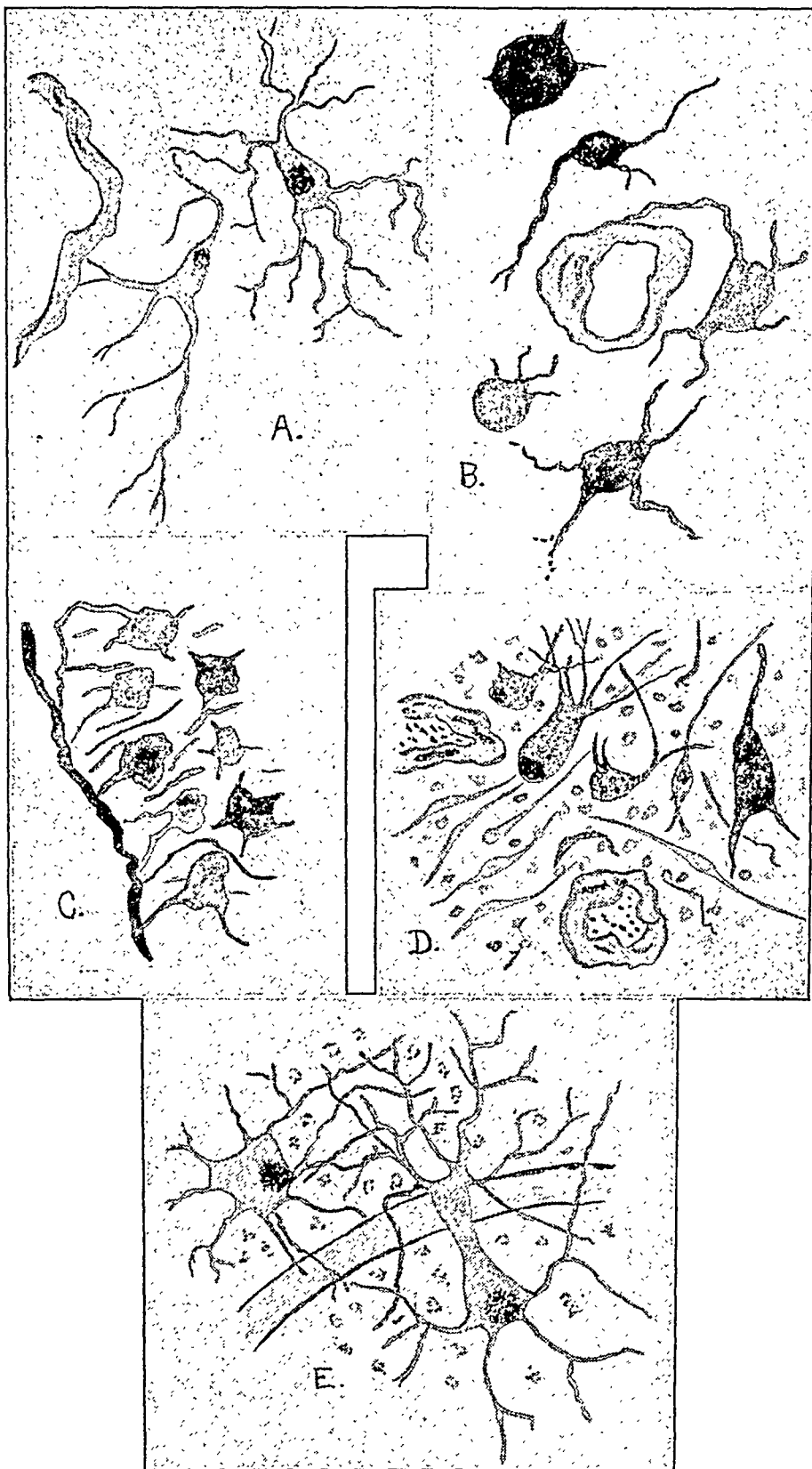
The study of the spongioblastomata is of great interest in this respect. Each tumour is different in certain respects, but certain fairly broad types of reaction may be observed. It must be noted that the adjacent grey matter seems productive of larger and more hypertrophic forms than the white. Hence the appearance at different parts of the edge of a single tumour may be variable.

Four general types of reaction were noted:

1. Although hypertrophic amoeba-like forms occur away from the tumour edge they rapidly become degenerate, poorly stained, and disappear. In this type of glioblastoma no inclusion forms are observed. Such an appearance may be noted at the edges of certain carcinoma nodules. It is presumed that in this type noxious products determine the death and disappearance of the astrocytes.

2. A transition between these hypertrophic forms and certain astrocytes found within the tumour forms a very definite feature. This was clearly seen in a tumour of one temporal lobe which was interpreted as an anaplastic ependymoma. The illustrations (Text-fig. 8) show a zone of deeply staining cells at the tumour edge. At the margin many of these cells are of amoeboid appearance, with short stubby processes, and the appearance is strongly suggestive in places of an area of so-called astroblasts (Text-fig. 8c). Within the tumour these same forms occur, but with many polar cells of the nature of ependymal spongioblasts and numerous unstained, undifferentiated forms. If the tumour edge had not been stained it could have been easily assumed that a spongioblastic tumour was differentiating into astrocytic forms. Another area of the tumour (Text-fig. 8E) presents an appearance less difficult of interpretation. Here we have well formed astrocytes associated with undifferentiated cells, without any suggestive transition forms.

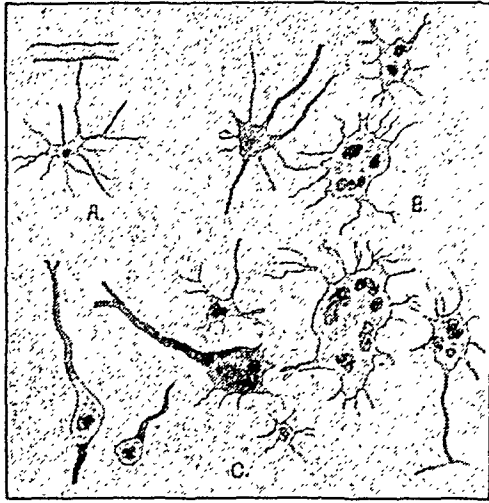
It must be noted that spindle and asteroid forms may occur together as neoplastic cells, and presumably divide at separate levels of differentiation. Cairns and Russell¹⁴ describe a spinal metastasis from a glioma in which both forms were indubitably neoplastic. Although they describe their tumour as a glioblastoma, they are not at all sure that it may not be an anaplastic astrocytoma. It must also be noted that similar cells may occur together in an ependymoma.



TEXT-FIG. 8

A. Astrocytes showing hypertrophy away from the tumour edge. B. Nearer the edge, showing deeply staining forms, and the loss of the fine ramifications. C. The amoeboid zone at the tumour edge, mimicking astroblasts. D. Within the neoplasm. Undifferentiated cells, with ependymal spongioblasts and large astrocytic forms of the inclusion type. E. A part of the tumour, an anaplastic ependymoma, far removed from the above. Only undifferentiated forms were present here with the large astrocytes.

3. In one tumour multinucleated giant cells are prominent. In ordinary haematoxylin and eosin sections these cells present all the appearances of foreign body giant cells (Fig. 34). In a deep section of the tumour edge stained by the gold sublimate method their origin from neuroglia is apparent (Fig. 35). As the edge is approached from the normal brain, ordinary fibrous neuroglia gives place to hypertrophied and often multinucleated forms. Deep within the tumour these forms are very evident, particularly in the vicinity of



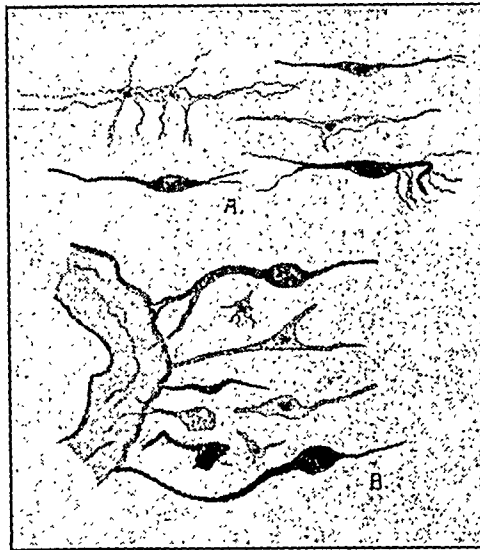
TEXT-FIG. 9

A drawing demonstrating the transition between tissue astrocytes, and giant astrocytes within a glioblastoma multiforme. A. An astrocyte just outside the zone of hypertrophy. B. Multinucleated astrocytes outside the tumour edge. C. Astrocytes of the inclusion type within the tumour.

vessels. Some of the cells are of enormous size, with the nuclei arranged at the periphery of the cell; other cells are small, almost of the size of the normal astrocyte; others again are of bizarre shape — club-shaped, flask-like, or polar (Figs. 36, 37, and Text-fig. 9). The tumour cells are undifferentiated and there do not seem to be any intermediate types between these and the deeply staining inclusion cells. In one part of the tumour there is a definite capsule of giant astrocytes. Their large fibres are mixed in a dense network. This area is cortical and corresponds to the wall of a degeneration cyst. A portion of the tumour wall taken in this vicinity would have conveyed the impression that an astrocytoma was being dealt with, but

comparison of this with other areas leaves no doubt that these cells were not neoplastic but reactive.

Although giant cells in a glial tumour may be and usually are neoplastic, in this particular example they are of the inclusion type. Considerable doubt is expressed by many writers as to the origin of the giant cells in the gliomata. They are considered by some to be true neoplastic cells, and by others to be included cells. Giant cells are usually neoplastic in origin, but many of the astrocytic and



TEXT-FIG. 10

A. Drawing of astrocytes outside the tumour edge of an undifferentiated glioblastoma. The piloid shape is assumed owing to the compression of a band of parallel nerve fibres. B. The same forms passing into the tumour as a perivascular cuff.

modified astrocytic forms encountered in the present series were undoubtedly derived from large reactive forms at the tumour edge.

4. The next example, the glioblastoma, presents a somewhat different appearance, the astrocytic forms being more confined to the larger vessels within the tumour (Figs. 8, 38, and Text-fig. 10). At the edge of this tumour are seen many forms, mainly piloid in character. The size of the processes is extremely variable and may be dense and highly stained, or fine and hair-like. The one cell may show both types of processes. Binucleated cells are present, and Text-figure 10A shows a cell with a constriction showing it to be

about to divide. The tumour substance contains closely packed undifferentiated cells and prominent vascular cuffs of an astrocytic nature. Not only is the appearance of these cells similar to that occurring in the reactive zone, but occasionally a vessel may be seen entering the tumour surface, surrounded by the same cuff, in continuity with the outer zone of astrocytes. The form of these perivascular cells reveals types that may be described as astroblastic, asteroid, or even spongioblastic. Yet they are derived from brain astrocytes and cannot be given an embryonal value.

The astrocytes observed within the medulloblastomata have usually been described as resulting from the process of differentiation. An area is shown in Figure 39. This method of derivation is inconceivable in this tumour. There are no suggestions of any intermediate forms, and no other deduction can be made but that they are non-neoplastic cells. In another example, where the tumour edge is clearly defined, their passage into the tumour can be traced. It is felt that it is insufficiently recognised that tissue astrocytes associated with the vascular system may form a stroma within these tumours. Further, in the one example of dissemination of a medulloblastoma within the subarachnoid space occurring in this series, no astrocytes can be observed in these tumours growing in a non-gliar tissue.

The astrocytoma presents a more difficult problem. The astrocytic reaction at the edge of many of these slowly growing tumours is not marked. At times, however, for no very apparent reason, it may be. Thus, in one cerebral astrocytoma, although diffuse in type, a well marked edge can be noted in one place at the margin of the grey matter. An increase in number and size of the normal astrocytes can be traced from without to the tumour edge. After that, in the tangle of forms, it is impossible to say which is tumour cell and which true astrocyte.

The current views on tumour formation do not foster the belief that the cells of invaded tissue may become neoplastic. We have then the possibility in an astrocytoma of a mixture of neoplastic and non-neoplastic astrocytes. Astrocytic cells at a tumour edge may, moreover, assume somewhat the form of tumour cells. Lhermitte and Oberling have noted the curious branching shape and volume of the astrocytes at the margin of tumours of the nature of the *astrocytome giganto-cellulaire*. It is possible that the same chemi-

cophysical factors may have caused uniform swelling in the cytoplasm of invading and invaded cells alike. This was extremely well shown in one example in this series.

The oligodendrogliomata frequently show many astrocytic forms and an interpretation of transition between these and oligodendroglia is frequently suggested. Kwan and Alpers expressed doubt in certain of their cases as such forms were more numerous near the margins of the tumours and near the vessels. In the one example in this series in which astrocytic forms occurred within the tumour there was no doubt that they were inclusion types. A well marked astrocytic reaction occurred at the margin, and was continued for a limited distance within the tumour. Moreover, no transition forms were apparent. This observation is merely recorded, and no attempt is made to deny that such transition forms do occur. The relation of oligodendroglia and astrocyte is too close to doubt that such a transition is not only possible but highly probable. It is, however, not a necessary assumption in every case.

Summary: 1. To the already varied picture of the glioblastoma multiforme further variations may be added by included astrocytes. Such cells may vary from the small astrocytic forms to huge multinucleated giant cells. In addition they may assume a polar form, or may present the appearance of astroblasts.

2. Such cells are particularly apt to be present near the edges of tumours and in the vicinity of vessels, although they are not confined to these areas.

3. The protoplasmic astrocytes of the grey matter are particularly apt to give origin to these larger forms.

4. It is possible that many of the variations in form of the brain astrocyte may be produced by such physicochemical factors as anoxaemia, degeneration products, the direction of stress, or the texture of the tissue.

5. Appearances may be present that suggest astrocytes may divide or attempt to divide within the tumour substance. They form there a type of stroma in the less degenerative areas.

6. In certain of the medulloblastomata an astrocytic stroma may be present. Such a stroma in the examples examined was certainly non-neoplastic.

7. Evidence is adduced that suggests certain of the astrocytomas may contain non-neoplastic mixed with the neoplastic astrocytes.

8. A similar stroma may occur in any of the gliomatous tumours in those areas in which degeneration is not prominent.

9. Many of these non-neoplastic astrocytes are introduced along with the blood vessels supplying the tumour. Occasionally they may form an actual cuff about such vessels.

CONCLUSIONS

One conclusion derived from this study is that names with an embryogenetic significance do not in all cases give the true values of the cells described. This in particular applies to the medulloblast and to certain of the spongioblasts and astroblasts. The neuroblast is also a cell that is particularly difficult to identify. It may be confused with included nerve cells, cells of the astroblast type, and even with certain anaplastic, rapidly subdividing cells.

The nature of a tumour will frequently be more correctly interpreted by the assumption of anaplastic changes overtaking a well differentiated tissue rather than ascribing it to differentiation of embryonal cells.

The medulloblastoma of childhood, however, possesses certain qualities that suggest the possibility of an embryonal origin.

The ordinary gliomata appear to fall into certain categories, as follows:

1. Tumours of adult tissue:
 - (a) Astrocytoma and its modifications.
 - (b) Ependymoma and its modifications.
 - (c) Oligodendroglioma.
 - (d) Pinealoma.
 - (e) Adult ganglioneuroma.
2. Highly anaplastic tumours which may occur independently or in association with any of the above (glioblastoma multiforme).
3. Transitional forms between the above:
 - (a) Astroblastoma.
 - (b) Polar spongioblastoma.
4. The medulloblastoma (possibly a true embryonal tumour).
5. Rare tumours of the type of medullo-epithelioma and neuro-epithelioma, concerning which no opinion is expressed here.

The mitotic figure content of such tumours offers the most ac-

curate index of their activity, rather than deductions derived from their resemblance to embryonal cells.

The difficulty in distinguishing between truly neoplastic cells and those that are derived from the glial tissue is discussed in some detail. The great advantage of postmortem material as opposed to operative fragments is here apparent.

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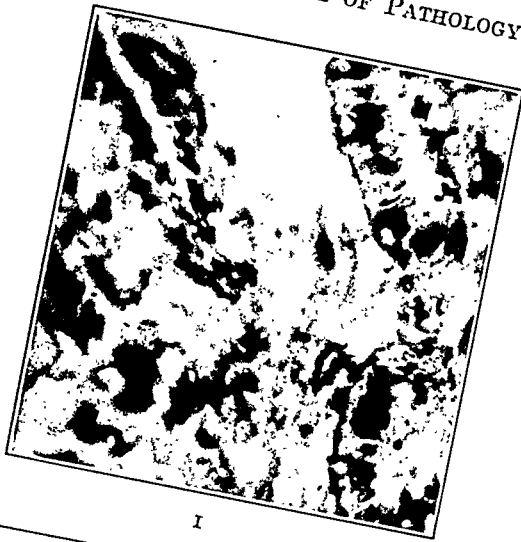
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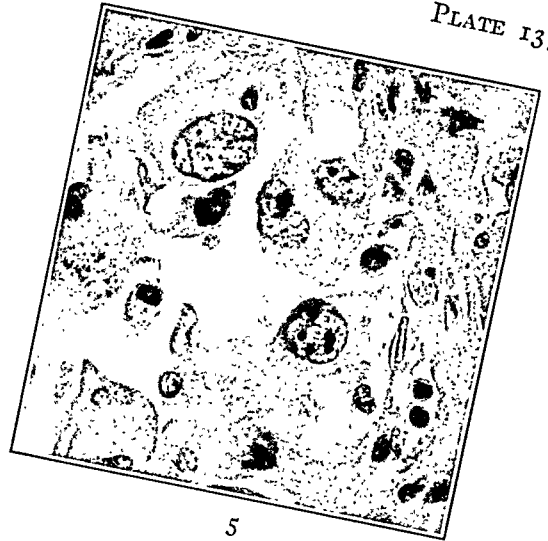
DESCRIPTION OF PLATES

PLATE 137

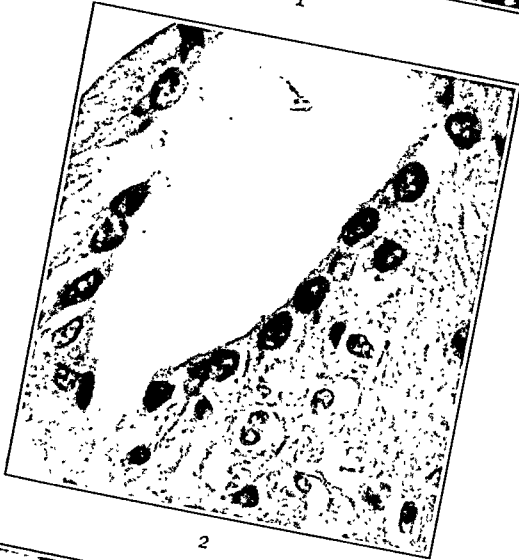
- FIG. 1. Section of a small, well defined tumour growing on the floor of the third ventricle. The convoluted bands of columnar cells suggest the medullo-epithelioma, yet the tumour showed little evidence of activity. Haematoxylin-eosin. $\times 350$.
- FIG. 2. An ependyma-lined cleft in a cerebellar ependymoma. Haematoxylin-eosin. $\times 480$.
- FIG. 3. So-called medulloblasts in a cerebellar medulloblastoma. Haematoxylin-eosin. $\times 750$.
- FIG. 4. Polar forms of a medulloblastoma occurring in a child's cerebrum. One cell is in mitosis. Haematoxylin-eosin. $\times 750$.
- FIG. 5. A frontal lobe tumour composed almost entirely of giant cells. Certain of the nuclei contain a single nucleolar-like mass, giving the cell somewhat the appearance of neuroblasts. The tumour contains numerous mitotic figures. Haematoxylin-eosin. $\times 450$.
- FIG. 6. Astroblasts in a glioblastoma multiform. Pyridine silver. $\times 750$.
- FIG. 7. Small unipolar cells (see Text-fig. 2c). Gold sublimate. $\times 750$.



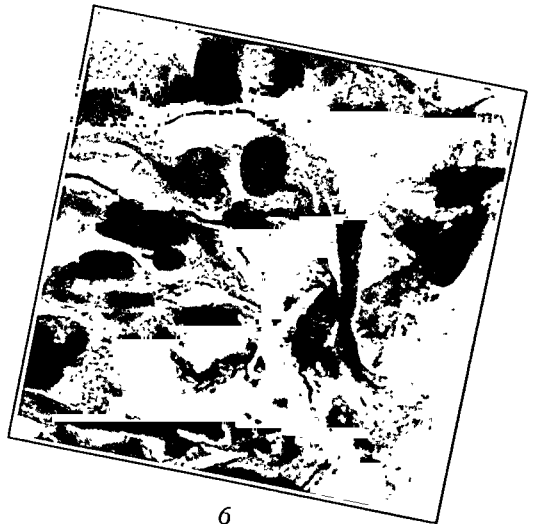
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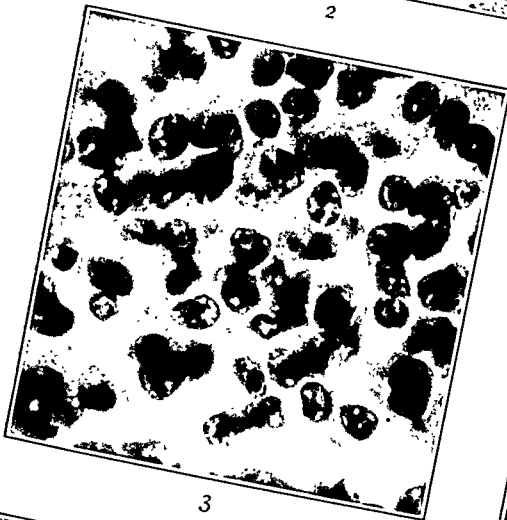
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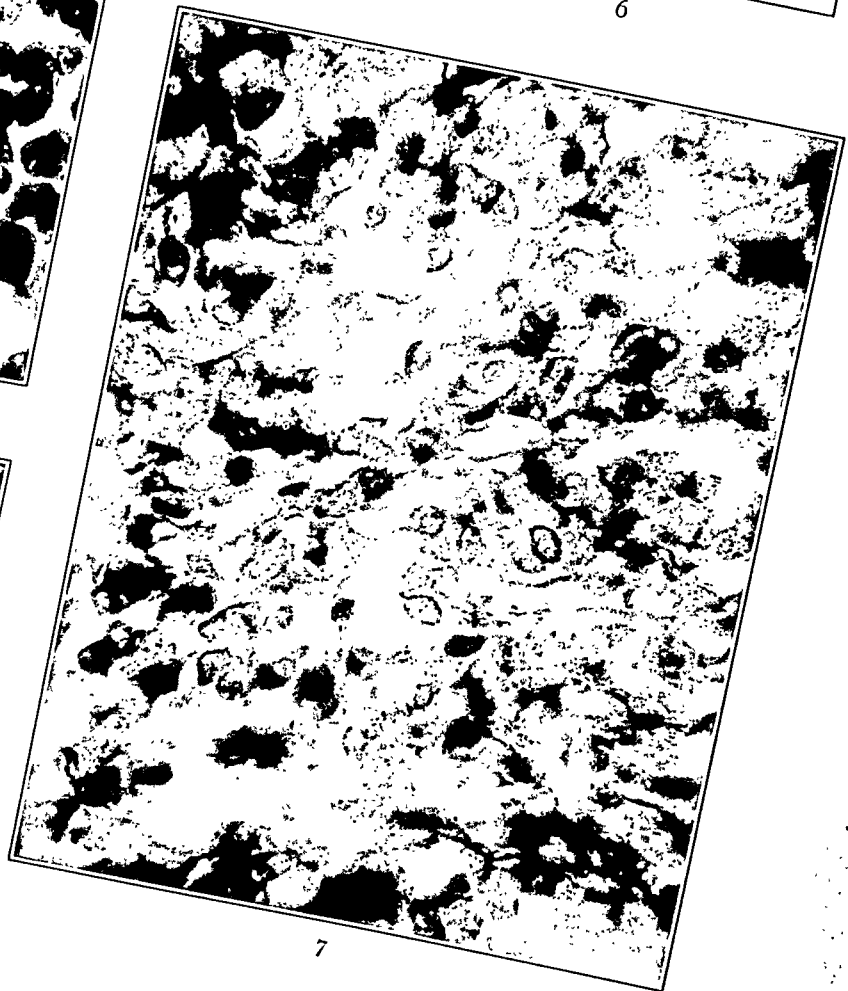
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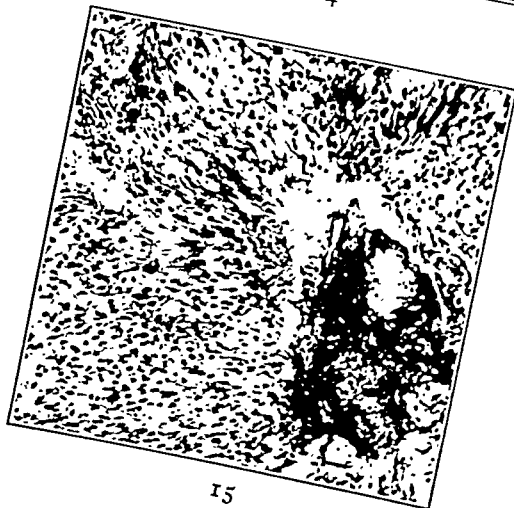
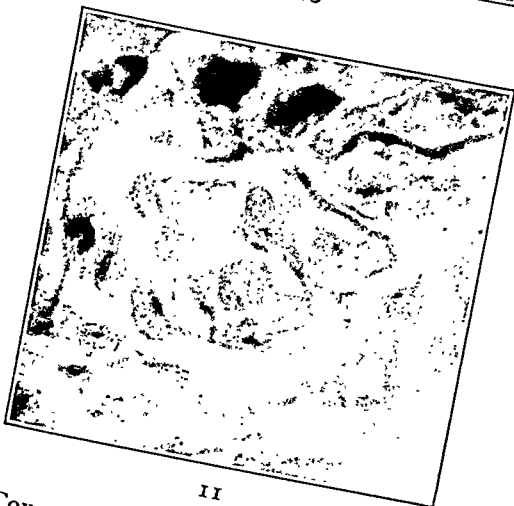
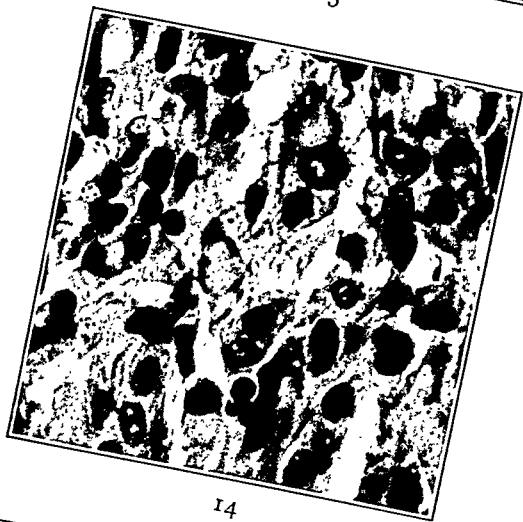
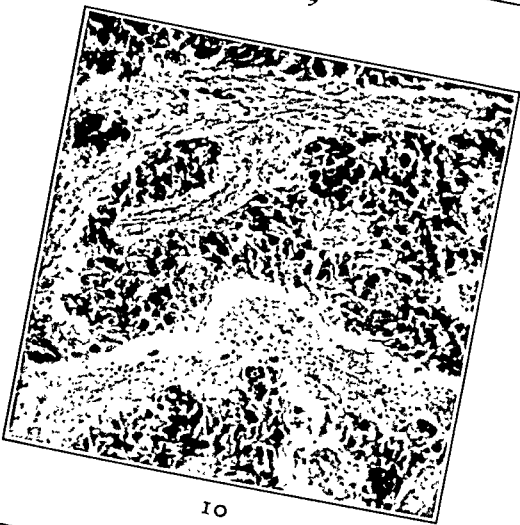
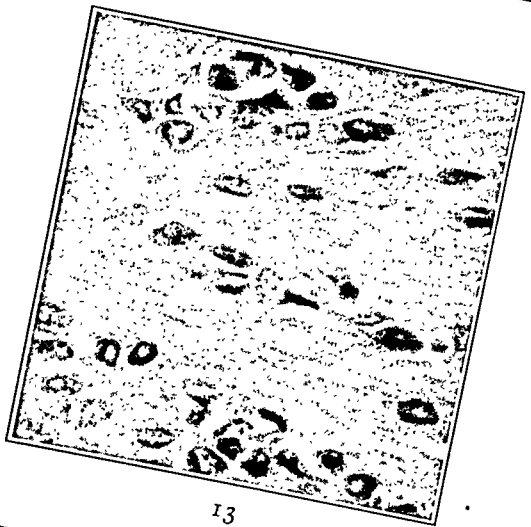
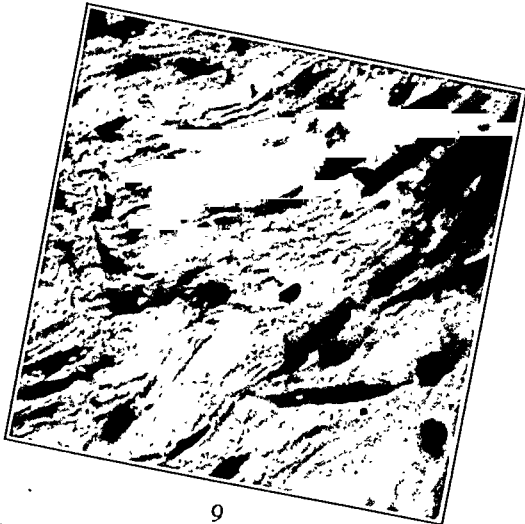
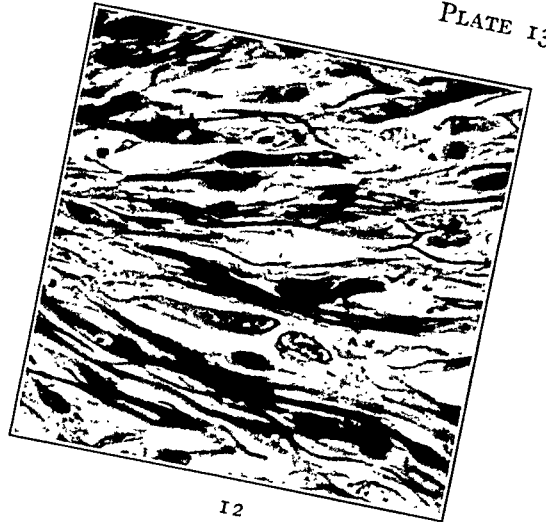


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Cox

PLATE 138

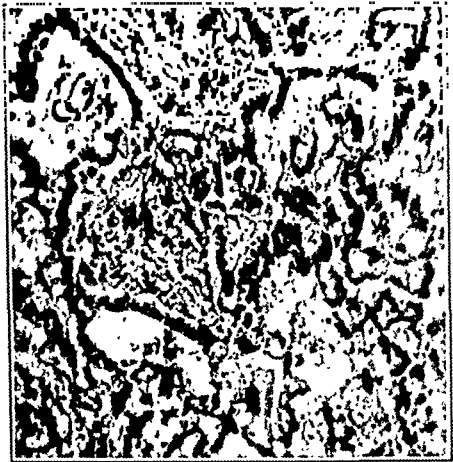
- FIG. 8. An astrocytic cuff within a glioblastoma multiforme. This is derived from the hypertrophied astrocytes of the tumour edge. Gold sublimate. $\times 60$.
- FIG. 9. Bipolar cells in a glioblastoma multiforme. Iron haematoxylin. $\times 400$.
- FIG. 10. An astroblastoma. The cells are enclosed by connective tissue bands, to which their processes are attached.
- FIG. 11. Individual cells of Fig. 10. Gold sublimate. $\times 450$.
- FIG. 12. Polar cells in a pontine tumour in a child of 4 years. Iron haematoxylin. $\times 480$.
- FIG. 13. A glioma of subependymal type. Haematoxylin-eosin. $\times 450$.
- FIG. 14. A deeper part of the same tumour, showing ependymal cells. Many of these were shown by the gold sublimate stain to be astrocytic. Haematoxylin-eosin. $\times 480$.
- FIG. 15. Arrangement of ependymal spongioblasts about vascular channels. Silver pyridine. $\times 90$.



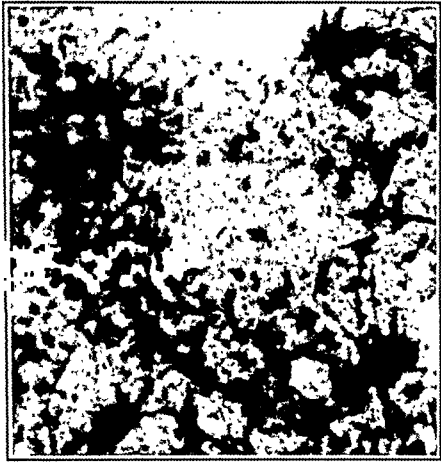
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PLATE 139

- FIG. 16. Ependymoma of neuro-epitheliomatous type associated with a cerebellar cyst. Haematoxylin-eosin. $\times 150$.
- FIG. 17. Oligodendroglioma, showing the interfascicular arrangement of the cells. Haematoxylin-eosin. $\times 450$.
- FIG. 18. Swollen oligodendroglial cells in an oligodendroglioma, producing the boxed-in effect. The vascular endothelium is prominent. Haematoxylin-eosin. $\times 300$.
- FIG. 19. Protoplasmic astrocytes. Gold sublimate. $\times 750$.
- FIG. 20. A cerebral astrocytoma. Gold sublimate. $\times 380$.
- FIG. 21. The anaplastic centre of the same tumour as in Fig. 20.
- FIG. 22. *Astrocytome, giganto-cellulaire*. The tumour was a small nodule occurring in the wall of a large cerebellar cyst in an adult. Haematoxylin-eosin. $\times 300$.
- FIG. 23. A malignant pinealoma. Mitotic figures are abundant. Haematoxylin-eosin. $\times 540$.



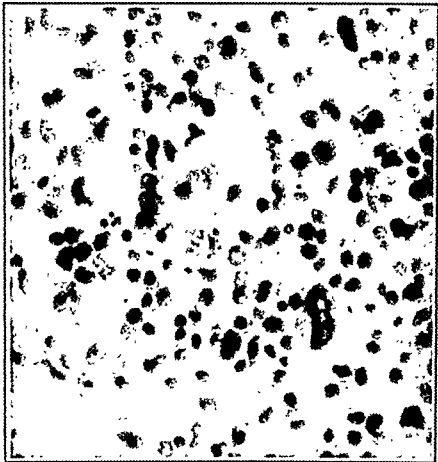
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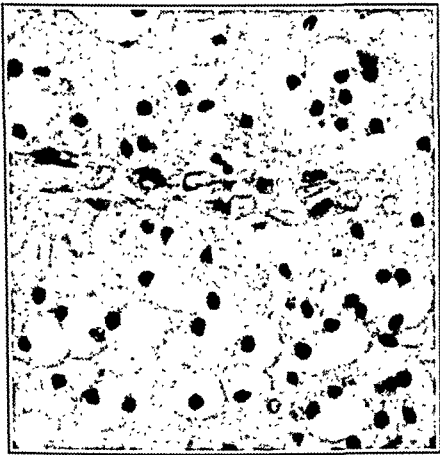
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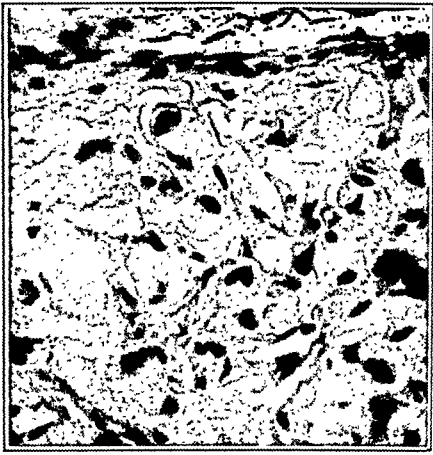
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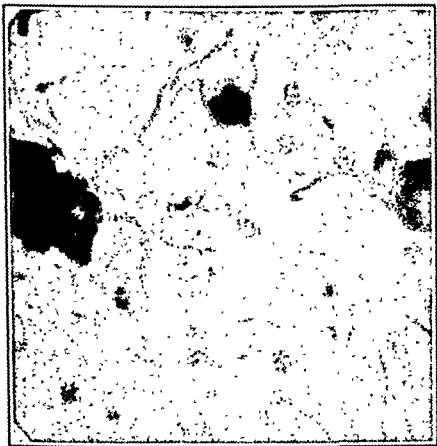
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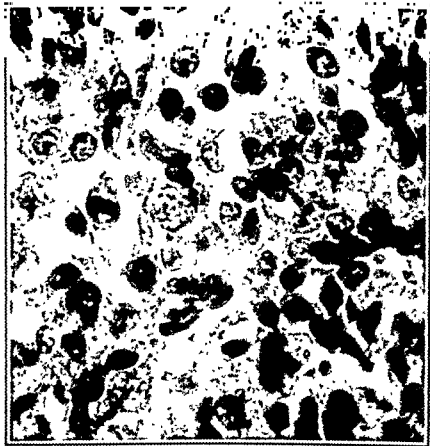
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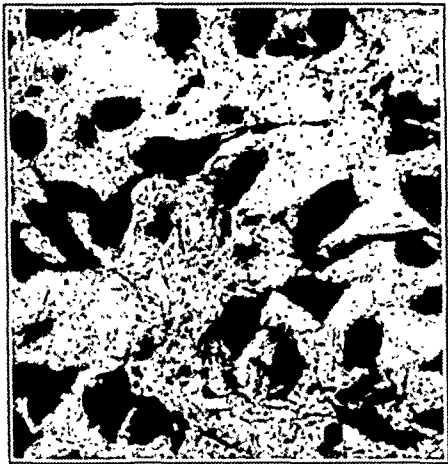
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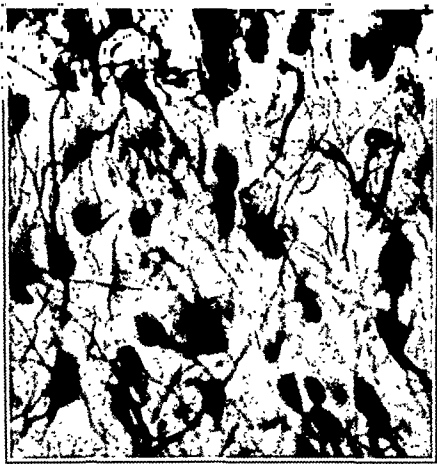
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PLATE 140

- FIG. 24. Cells in a so-called ganglio-glioma of a frontal lobe. Pyridine silver. $\times 400$.
- FIG. 25. The cells of an astroblastoma showing how the nuclei may mimic those of nerve cells. Haematoxylin-eosin. $\times 480$.
- FIG. 26. Neuroblast-like cells occurring in a malignant tumour arising in the frontal lobe of a child. Haematoxylin-eosin. $\times 480$.
- FIG. 27. Illustrating the neuroblast-like appearance that may be assumed by the nuclei of a rapidly growing tumour. The section is that of a sarcoma of the femur.
- FIG. 28. Nerve fibres occurring deep within a cerebral astrocytoma of slow growth. The processes of the astrocytes are also faintly impregnated. Pyridine silver. $\times 400$.
- FIG. 29. Nerve cells and nerve fibres within a highly cellular glioma of the glioblastoma multiforme type. Silver carbonate. $\times 500$.
- FIG. 30. Nerve cells within a tumour removed from a child's cerebellum. The section closely mimicked the adult ganglio-neuroma. Silver carbonate.
- FIG. 31. Degenerating nerve cells from the tumour shown in Fig. 30.



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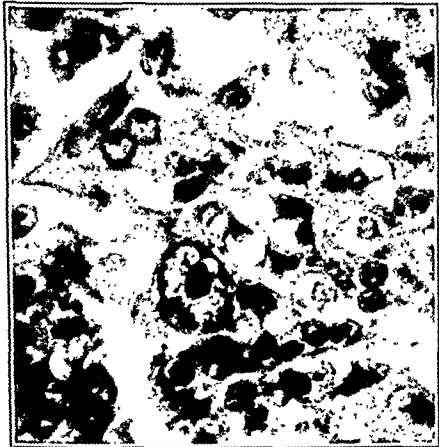
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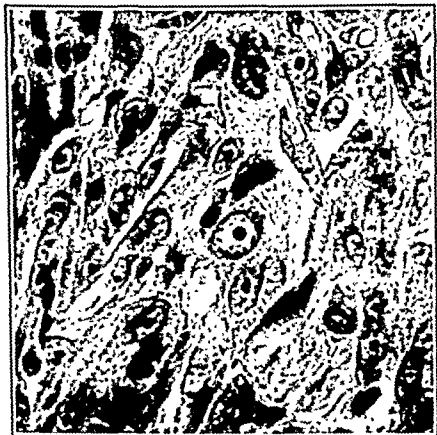
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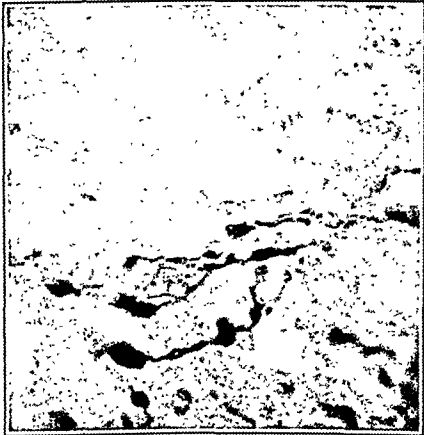
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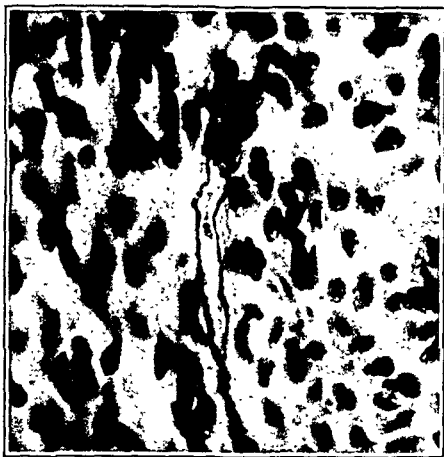
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PLATE 141

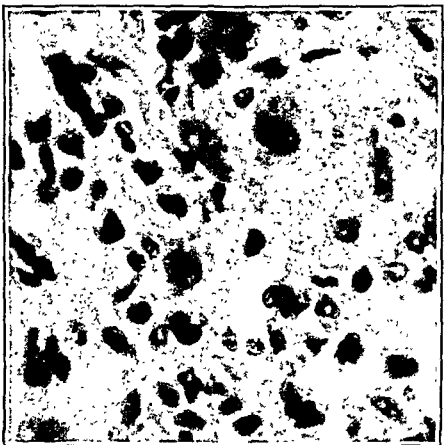
- FIG. 32. Nerve fibres deep within a slowly growing cerebellar medulloblastoma. Pyridine silver. $\times 480$.
- FIG. 33. Nerve cells deep within a medulloblastoma. These were derived from a narrow strand of enclosed tissue. Haematoxylin-eosin. $\times 380$.
- FIG. 34. Giant cells in a glioblastoma multiforme. Haematoxylin-eosin. $\times 150$.
- FIG. 35. A similar portion to Fig. 34, showing giant astrocytes. Gold sublimate. $\times 150$.
- FIG. 36. Giant astrocytes outside the edge of the same tumour. Gold sublimate. $\times 380$.
- FIG. 37. The same forms as inclusion cells at the margin of a vessel deep within the tumour. The transition could be clearly traced. Gold sublimate. $\times 380$.
- FIG. 38. A higher magnification of part of the astrocytic cuff. Gold sublimate. $\times 400$.
- FIG. 39. Astrocytes deep within a medulloblastoma. Gold sublimate. $\times 480$.



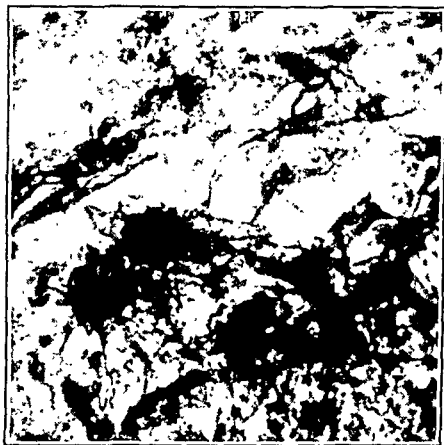
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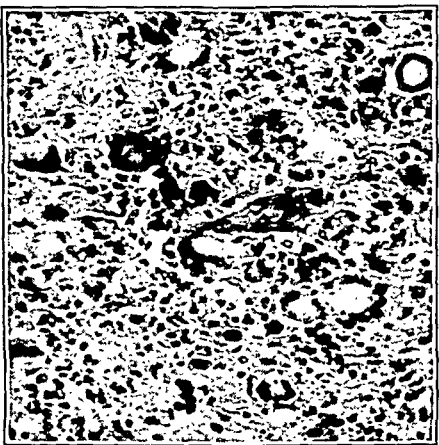
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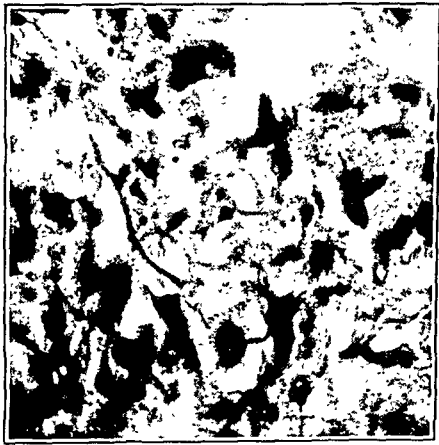
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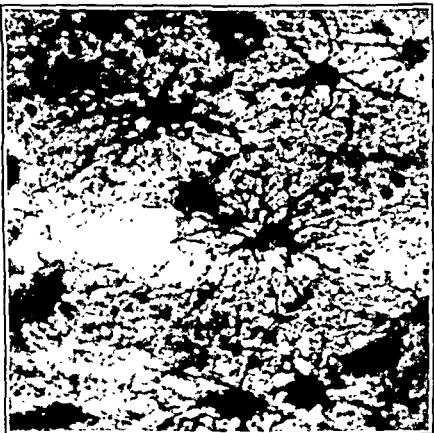
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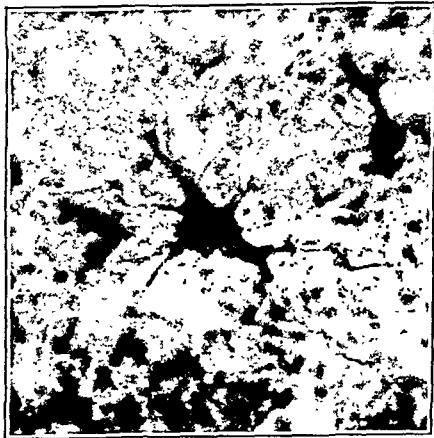
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THE ORIGIN OF ONE TYPE OF SECONDARY PNEUMONIA *

VIRGIL H. MOON, M.D.

(From the Department of Pathology, Jefferson Medical College, Philadelphia, Pa.)

This discussion pertains to the development of pneumonia following pulmonary congestion and edema. Moon and Kennedy¹ have shown that marked congestion and edema, associated with capillary hemorrhages and increased concentration of blood, are found regularly in shock. The evidence is strong that such changes constitute the essential pathology of that type of circulatory failure. The capillary phenomena are most marked in the respiratory tract and result from atony of the capillaries and venules. Dale, Laidlaw and Richards² showed that histamine shock results from atony of capillaries and venules and Krogh³ showed that capillary atony, increased permeability and edema are the characteristic effects of a wide variety of capillary poisons. Circulatory failure of this type is manifested clinically by a progressive decline in blood pressure, rapid respirations, rapid weak pulse, a decline in temperature and an increased concentration of blood. Pulmonary congestion and edema follow. Clinicians usually assign such circulatory failure to cardiac weakness, even though postmortem examination shows no gross or microscopic changes in the cardiac structure.

Circulatory shock or collapse develops characteristically following burns and other severe intoxications. Atchley⁴ cited cases of diabetic acidosis and severe acute infections in which the shock syndrome was present. He considered these cases to be due to the same mechanism as in traumatic shock. He also reported a case of rattlesnake bite where the venom probably was injected directly into a vein. Characteristic shock-like manifestations resulted.

Krogh³ believes that capillary atony results from various forms of toxemia, and produces circulatory shock in diverse clinical conditions. I have had occasion to substantiate this view by post-mortem studies in such cases.⁵ These have included intestinal obstruction, toxemias of pregnancy, burns, infections of unusual

* Aided by a grant from the Martin Research Foundation.

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severity, intestinal perforations, hemorrhagic pancreatitis and poisoning with various drugs.⁶ In each instance the gross and microscopic changes were identical with those present in traumatic shock, and included marked congestion and edema of the lungs. If the capillary atony and resulting deficiency of circulation are of marked degree the patient dies of circulatory failure with shock-like manifestations. If the circulatory deficiency is less extreme, but is sufficient to produce moderate pulmonary congestion and edema, the development of secondary pneumonia frequently occurs.

In lungs where the circulation is sluggish, capillaries and venules engorged, and where the alveoli contain albuminous fluid, ideal conditions are present for the development of infection. Various pathogenic organisms are present in the respiratory passages of the majority of persons, and fluid resulting from edema provides an excellent culture medium for their growth. Infection of the lung developing in this manner presents characteristics not found in primary lobar or lobular pneumonia. The lungs have a deep purplish red color (Fig. 1), are heavy, edematous, and contain irregular areas of consolidation which do not conform to the distribution of the bronchial branches. These areas vary greatly in size, distribution and number and are most numerous in the dependent portions. Frequently there are scattered, imperfectly consolidated areas about 1 cm. in diameter. In other cases larger irregular patches occur. The character of the consolidation varies with the causative organism, the rapidity of the spread of the process and with the time elapsing before death. Occasionally the areas become confluent and resemble lobar pneumonia somewhat, except that they are mottled and mosaic-like in appearance and their density is less uniform. Bloody frothy fluid escapes from cut surfaces and from the smaller bronchi when pressure is applied. The bronchial mucosa is swollen and cyanotic in color. The pleural surfaces contain numerous minute areas of capillary hemorrhage. There are scattered areas of hemorrhage throughout the lung substance, and occasionally larger areas resembling hemorrhagic infarcts are seen.

Microscopic examination shows areas of leukocytic infiltration which varies greatly in density (Figs. 4, 5, 6 and 7). The capillaries and venules are dilated and engorged. Considerable fluid is present containing varying numbers of red cells from capillary hemorrhages. There is a deficiency of fibrin and the consolidation is less dense than

in primary pneumonias. The results of bacteriological examination are inconstant. Various organisms are found and cultures usually contain more than one type. Pneumococci, streptococci, staphylococci and *Micrococcus catarrhalis* predominate. Occasionally *B. influenzae* and other bacteria are present.

Secondary pneumonia following burns is typical of the pneumonias that develop by the shock syndrome. The following case is illustrative. Three men were seriously burned following a collision in which a gasoline tank burst and was ignited. The burns were of the second and third degree, involving chiefly the legs, arms and face. Shock was a marked clinical feature in each case, but varied with each individual. The burns varied somewhat in the amount of skin involved. The man having the most extensive burn had a red cell count of 8,360,000 and the hemoglobin was 120+. The Dare hemoglobinometer which was used had no provision for higher readings. He died within forty-eight hours and the postmortem findings were typically those of shock. No pneumonia was present.

The second victim, whose burns were slightly less extensive, died of secondary pneumonia eleven days later. His condition of moderate shock, as shown by blood pressure, blood concentration and other clinical features, continued during the eleven days. At postmortem the lungs showed the characteristic features described for this type of pneumonia (Fig. 7).

The third man who was burned recovered. His condition of shock was less in degree and gradually subsided. The gradual recovery from shock is well illustrated by the changes in blood concentration on consecutive days:

Date	Specific gravity	Hemoglobin	Red cells
Dec. 14	1.075	120 +	6,700,000
" 15	1.070	112	5,400,000
" 16	1.063	110	5,000,000
" 17	1.070	116	5,200,000
" 18	1.068	115	5,100,000
" 19	1.066	110	4,990,000
" 20	1.062	106	4,900,000
" 21	1.055	99	4,630,000
" 22	1.048	74	3,530,000
" 24	1.045	87	3,820,000

In material from 100 autopsies on adults secondary pneumonia occurred in sixteen cases. Congestion and edema of cardiac and renal origin were not included. Eight cases occurred following surgi-

cal procedures and one case each occurred in the following conditions: ulcerative colitis, acute hemorrhagic pancreatitis, drug poisoning (sedormide), obstructive jaundice, eclampsia, superficial burn of skin, diabetes with acidosis, and peritonitis. These presented the same type of pneumonia and were preceded by pulmonary congestion and edema in each case.

There is evidence that certain cases of pneumonia following influenza belong in this group. Many regard influenza as a systemic infection similar to measles and other exanthemata whose etiology has not been established. The severity of the disease in different epidemics and in different cases varies extremely. During the pandemic of 1918 in many of the most severe cases the individual died within two or three days of the onset of illness. These cases presented evidence of shock-like circulatory failure. There was a marked decline in blood pressure, weak pulse, rapid respirations and a decreasing temperature. Increased concentration of the blood was a prominent characteristic. Underhill and Ringer⁷ made blood studies in forty-three cases. In the sudden, severe cases there was a marked concentration of blood with hemoglobin values ranging from 110 to 140. Death resulted in each instance. No concentration of blood was present in the less severe cases and there was no history of it among those who recovered. They concluded that the degree of blood concentration was an index of the severity of the illness, which was especially valuable in prognosis. They noted that the lungs in the fulminating cases were indistinguishable from those following lethal gas poisoning, and I have found them indistinguishable from the lungs following fatal burns. Postmortem examination showed wet bloody lungs and extreme capillary congestion of the mucosa of the pulmonary and gastro-intestinal tracts. There were numerous petechial hemorrhages in the mucous, serous and endocardial surfaces. Blood-tinged fluid was present in pleural and pericardial cavities. The lungs were increased 200 to 400 per cent in weight, due to marked edema and congestion. In the most severe cases no pneumonia was demonstrable, either grossly or microscopically. Apparently death took place from circulatory failure before the development of pneumonia occurred.

In cases of somewhat less severity the individuals lived from seven to fifteen days. On postmortem examination congestion and edema were not so extreme but were marked. Pneumonia of irregular type

and distribution was the characteristic feature. The lungs were heavy, wet and bloody and the consolidation varied greatly in character and extent. Bacteriological examination in such cases gave varying results. Organisms of the pneumococcic-streptococcic group occurred most frequently. In this particular the pneumonia following influenza does not differ from the group of pneumonias under consideration (Fig. 3). The view is widely accepted that it is a secondary infection, but the importance of capillary atony and resulting circulatory inefficiency in its development has not been emphasized. I have compared the gross and microscopic features of the lungs following influenza with those of secondary pneumonia following burns, and find no essential differences. It seems probable that their mechanism of origin is similar.

SUMMARY

Various clinical conditions may result in a gradual failure of the circulation, which is not cardiac in origin but results from atony of the capillaries and venules. They include circulatory deficiency following extensive surgery, burns, various intoxications both metabolic and external in origin, abdominal conditions such as intestinal obstruction, pancreatitis, perforations, and severe systemic infections.

Marked pulmonary congestion and edema are characteristic features in these cases and increased concentration of blood is regularly associated with them.

One type of secondary pneumonia results from infection of such lungs with various organisms present in the respiratory tract.

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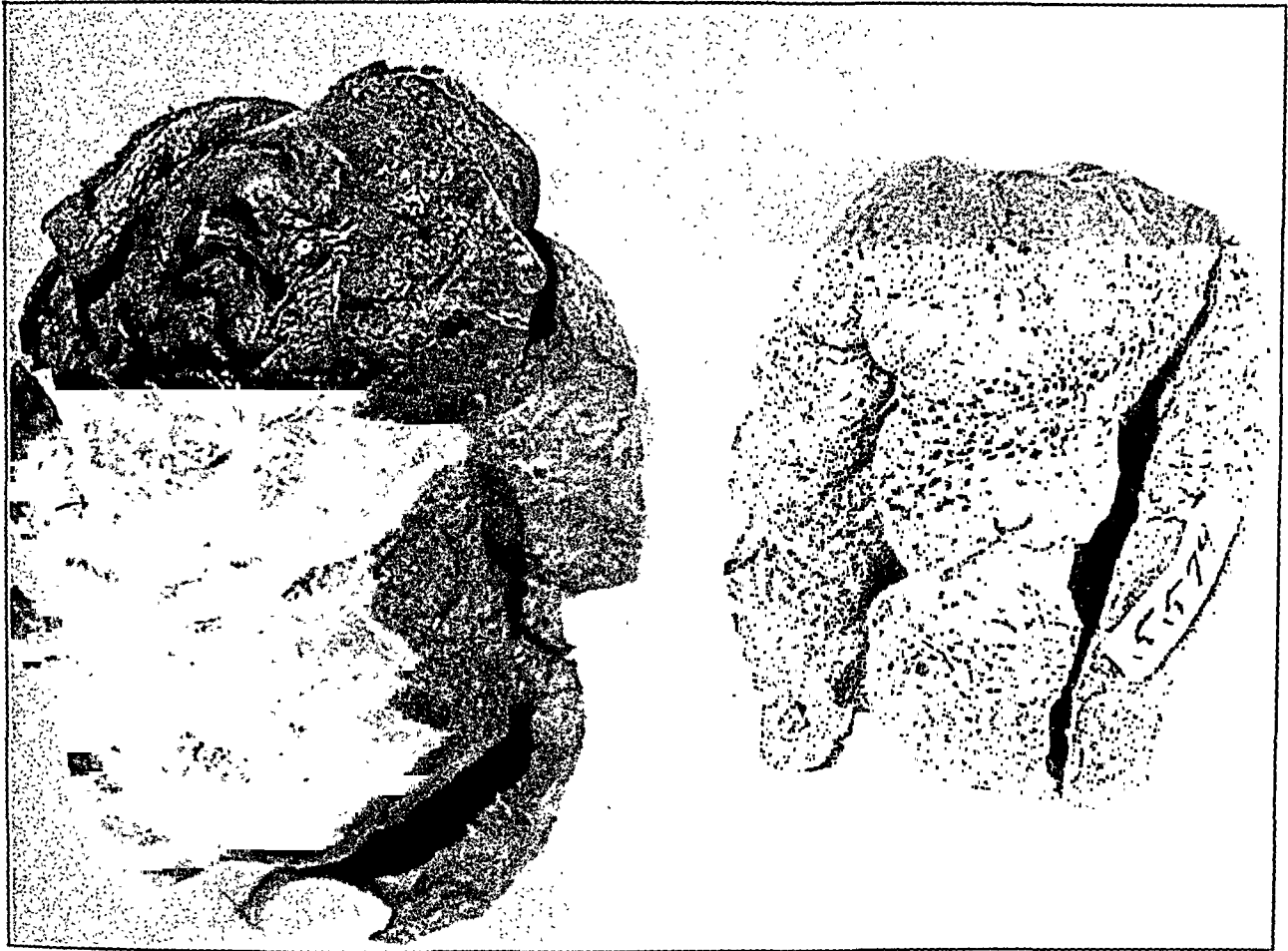
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DESCRIPTION OF PLATES

PLATE 142

- FIG. 1. Lung from a case of secondary pneumonia following drug poisoning. A man took an unknown quantity of sedormide. Profound narcosis was followed by circulatory failure ending in death four days later. Circulatory changes characteristic of shock, and irregular pneumonia in an early stage were shown at autopsy. Photographed with a normal lung (right) for comparison.
- FIG. 2. Photomicrograph of lung from the same case. Edema and early pneumonia are present with marked dilatation and engorgement of capillaries and venules with capillary extravasation. A small amount of fibrin is seen, less than is found in primary pneumonias. $\times 500$.
- FIG. 3. Another field from the same lung as in Figs. 1 and 2, showing bacteria in the edema fluid. At the point marked X a dilated capillary has ruptured and red cells are escaping. Gram-Weigert stain, formalin fixation. $\times 1200$.



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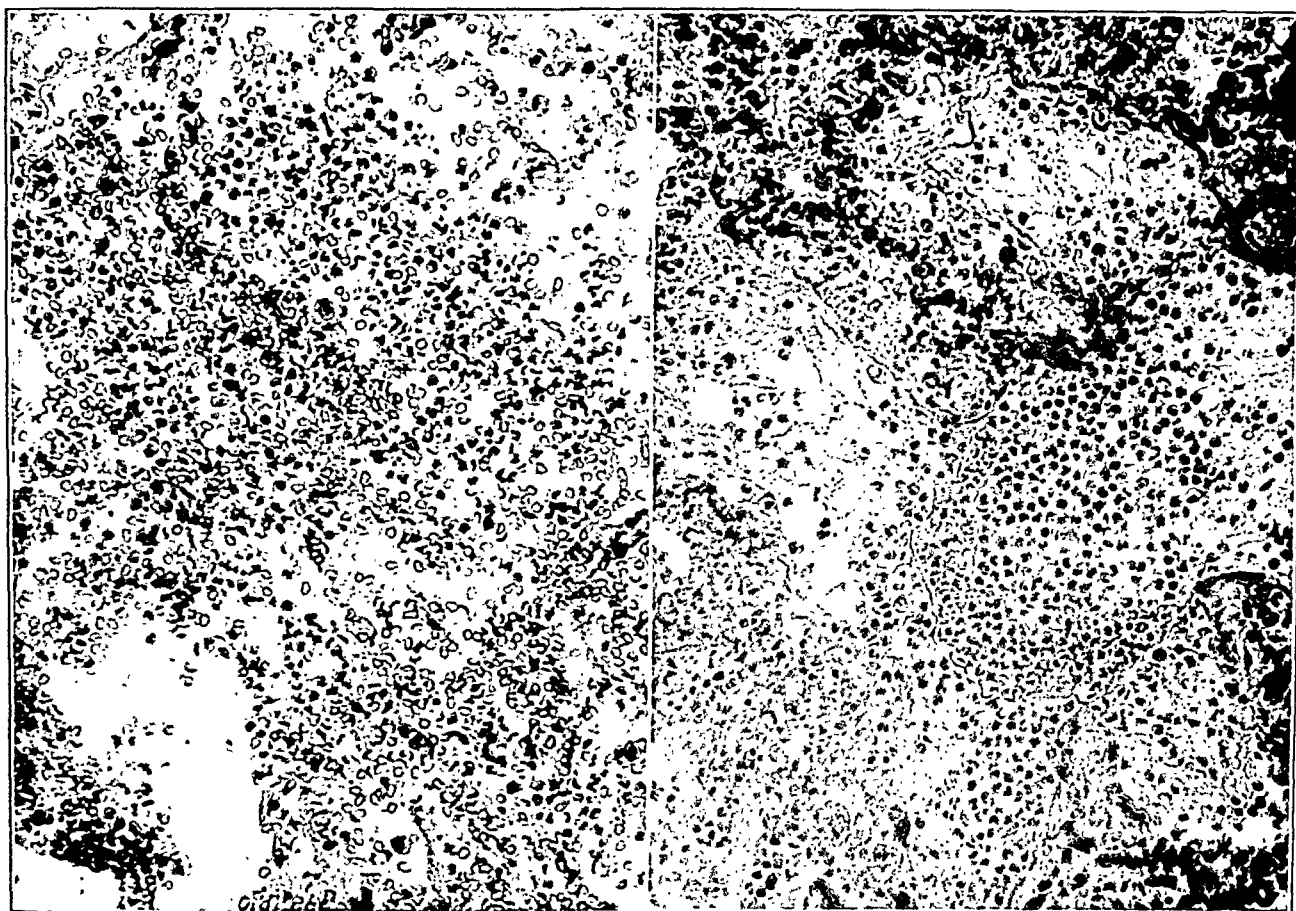
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Moon

Origin of One Type of Secondary Pneumonia

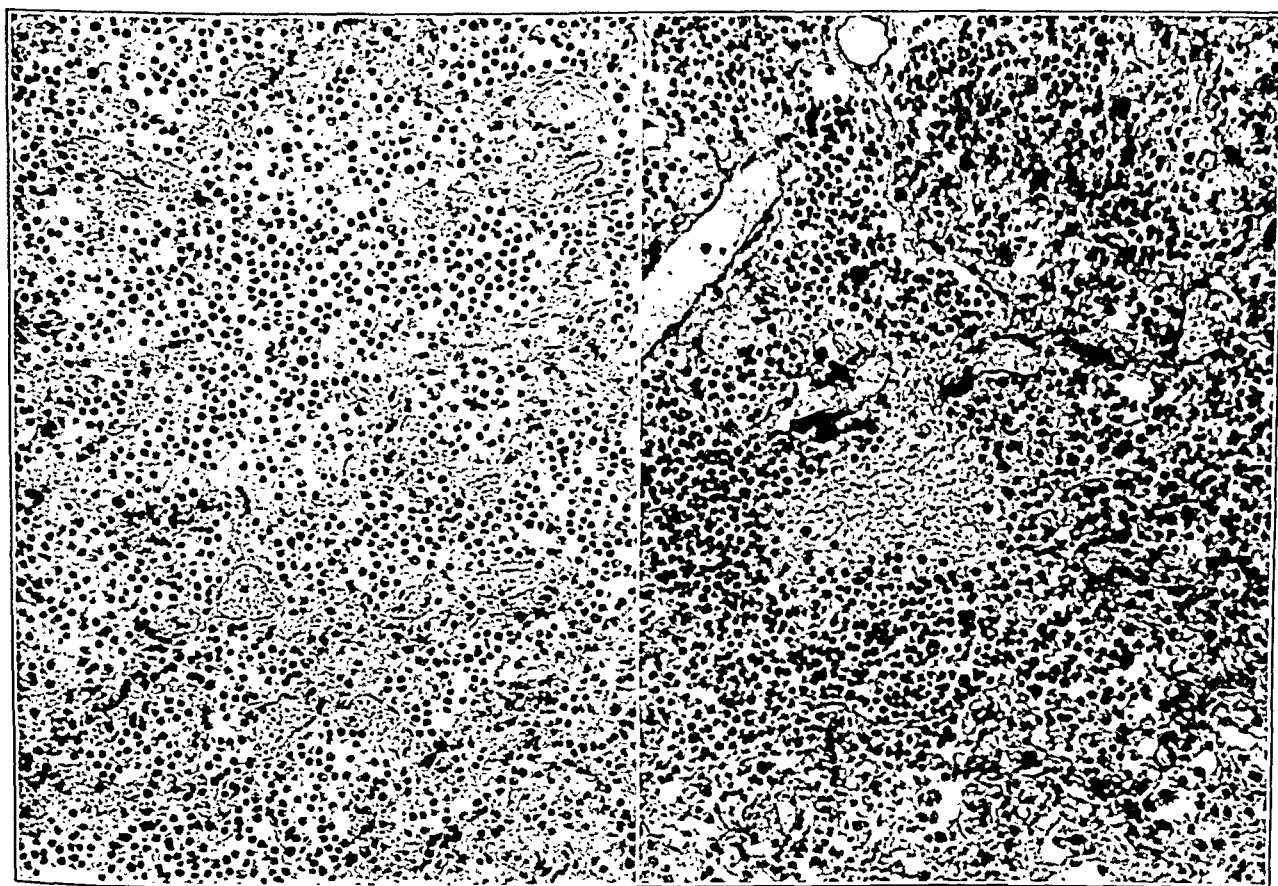
PLATE 143

- FIG. 4. Early pneumonia from a case where the shock syndrome developed following mesenteric thrombosis. $\times 500$.
- FIG. 5. A more advanced stage of pneumonia from a case in which circulatory failure developed during a severe toxemia of pregnancy. $\times 500$.
- FIG. 6. An area of pneumonia in an engorged edematous lung from a case of severe influenzal infection. $\times 500$.
- FIG. 7. Pneumonia in an advanced stage. In this instance death took place from secondary pneumonia eleven days after an extensive superficial burn. The blood vessels are packed with corpuscles. In the center of the field is a capillary hemorrhage. $\times 500$.



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Moon

Origin of One Type of Secondary Pneumonia

FIBROMA OF THE BREAST *

BÉLA HALPERT, M.D., AND DAVID L. DIAL, M.D.

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Benign neoplastic proliferations of the epithelial elements of the breast seem to furnish an incentive for neoplastic growth of the stroma. The name fibro-adenoma adequately expresses the structure of the resulting growth. The adjectives "pericanalicular" and "intracanalicular" further denote the relation of the component tissue elements. While fibro-adenoma of the breast is quite common, pure fibroma, *i. e.*, one containing no neoplastic epithelial elements, is rare. In fact, the case reported here is one of the few ever recorded.

REPORT OF CASE

Clinical History: Miss I. J. M., 47 years of age, a dietitian, was admitted to the New Haven Hospital on Sept. 15, 1932, complaining of a lump in the left breast. She stated that a small painless growth had been removed from the same breast in 1927. Subsequently a mass about 4 cm. in diameter recurred in about the same location underneath the skin 5 cm. above and lateral to the nipple, which was excised in May 1930. The microscopic structure of this growth was that of a fairly typical intracanalicular and pericanalicular fibro-adenoma of the breast (Fig. 1).

Several months prior to the present admission she again noted in the same breast a lump that gradually increased in size and was not tender or painful.

The patient appeared well nourished and apparently in good health. The systolic blood pressure was 120 and the diastolic 70 mm. Hg. The Kahn blood test was negative. The red blood cell count was 4,100,000 with 83 per cent hemoglobin, the white blood cell count was 8200, of which 69 per cent were polymorphonuclear leukocytes and 19 per cent lymphocytes. The urine contained no albumin or sugar.

In the upper outer quadrant of the left breast there was a firm, freely movable, well circumscribed mass measuring about 6 cm. in diameter. It did not involve the nipple and was neither fluctuant nor tender. The axillary lymph nodes were not enlarged.

On Sept. 16, 1932 the tumor was excised through an incision encircling the old operative scar. The gross appearance of the tumor on cut section resembled that of a fibro-adenoma, although the frozen section disclosed no epithelial elements. The operation was concluded by a conservative amputation of the breast, including the pectoral fascia.

* Received for publication June 8, 1933.

The specimen thus consisted of the tumor and the breast. The tumor presented a sharply demarcated, oval, firm, elastic, lobulated mass measuring 4 by 5 by 6 cm. It cut with increased resistance and a gritty sensation, disclosing smooth, glistening, pearly white surfaces with a pattern of interlacing strands and whorls. It contained no glandular or adipose tissue. The breast tissue proper measured 6 by 8 by 10 cm. The nipple and areola appeared intact. No masses were felt or seen anywhere.

Microscopic preparations stained with hematoxylin-eosin, Mallory's aniline blue, Mallory's phosphotungstic acid hematoxylin, and the Van Gieson method show the tumor to be composed of a fairly vascular, rather loose fibrous connective tissue containing no epithelial elements. It is surrounded by a thin but definite connective tissue capsule which separates the tumor from the surrounding normal breast tissue (Fig. 2). The cell nuclei and the fibrils run in interlacing streams and whorls. In some areas the connective tissue is as loose as embryonal tissue, in others it is fairly dense. The cell nuclei are spindle-shaped and are not hyperchromatic. Mitotic figures are scarce. No areas of necrosis are noted anywhere in the preparations.

The wound was nearly healed when the patient was discharged on Sept. 23, 1932. When last seen, eight months following operation, there was no evidence of recurrence of the growth.

COMMENT

In his monograph Williams¹ states that among the 2397 cases of mammary neoplasms in women analyzed by him there was one instance of this kind, a pure fibroma of the breast. He cites a specimen from Péan's collection, quotes from the pathological catalogue of the Hunterian Museum the description of three more specimens, and adds that Broca mentions having seen two cases of fibroma in the mammary region.

Suzuki,² in 1913, observed a tumor the size of a child's fist in the breast of a 43 year old woman. The tumor showed in parts the structure of a soft fibroma and in others that of a hard fibroma, and contained no glandular elements.

The next and most recent report of a case of pure fibroma of the breast also came from Japan and was made by Kon,³ in 1931. The patient, a 33 year old housewife, mother of five children, noticed a

small tumor in the upper portion of the left breast six years prior to admission. This gradually enlarged to reach the size of an infant's head, but remained well circumscribed and movable. The breast was removed, together with the axillary lymph nodes. The tumor presented firm, uniform, fibrous, whorled, gray-white cut surfaces. Microscopic preparations show it to be composed of interlacing bundles of fibrous connective tissue containing no epithelial elements. There was no recurrence of the growth three years following operation.

Our case is of interest in that the breast on two previous occasions was the site of neoplastic growth. The structure of the tumor removed at the first operation we do not know. The growth removed at the second operation was a fibro-adenoma. The growth removed at the third operation was a pure fibroma in which the connective tissue closely resembled the connective tissue elements in the second growth. The question naturally arises whether there is any causal relation between these growths or whether they are independent tumors arising at different times in the same breast. The latter seems to be the more plausible explanation since there is no morphological evidence that any of these growths were malignant.

SUMMARY

A pure fibroma of the breast, one of the few ever recorded, is reported. It occurred in a woman 47 years of age. The same breast on two previous occasions was the site of neoplastic growth. The structure of the tumor removed at the first operation is not known. The growth removed at the second operation was a fibro-adenoma. The one removed at the third operation was a pure fibroma in which the neoplastic tissue closely resembled the stroma of the second growth.

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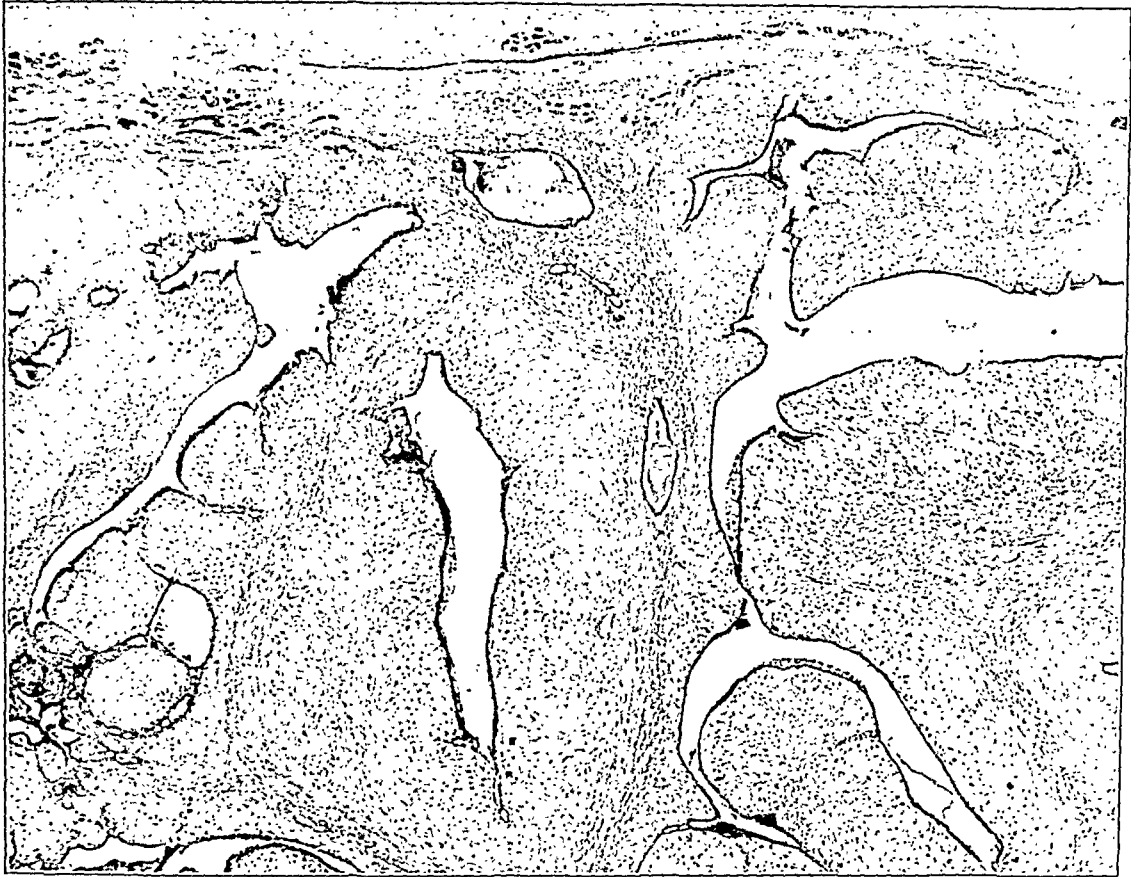
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DESCRIPTION OF PLATE

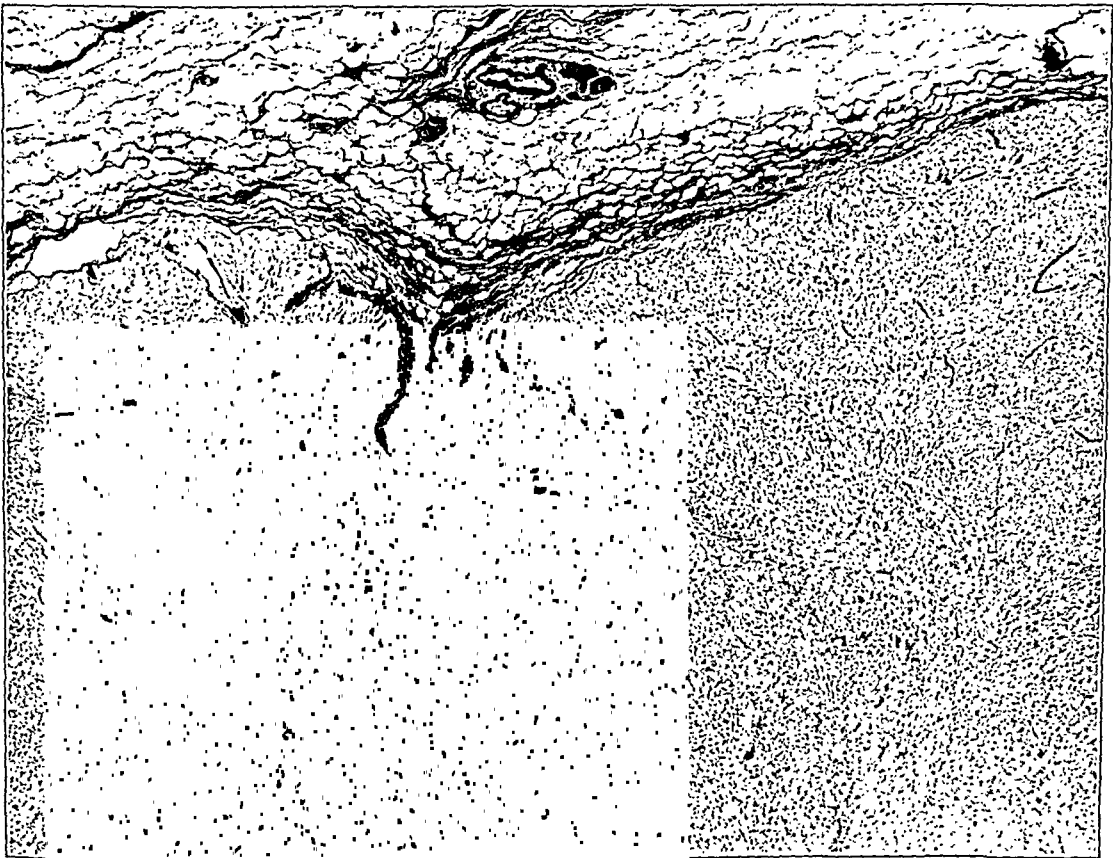
PLATE 144

FIG. 1. Fibro-adenoma of the breast, pericanalicular and intracanalicular, removed at the second operation in 1930. $\times 25$.

FIG. 2. Fibroma of the breast removed at the third operation in 1932. A thin but definite connective tissue capsule separates the tumor from the surrounding normal breast tissue. $\times 25$.



1



2

LIPOSARCOMA *

REPORT OF A CASE WITH INTRACRANIAL METASTASES

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In a recent report of three liposarcomas apparently originating in bone Stewart ¹ was scrupulously careful to avoid dogmatism in regard to the histological classification. He felt, however, that inasmuch as the lipoblast was the only cell within the bone marrow not represented in the group of primary tumors it was reasonable to expect that liposarcoma would occur in this location. Moreover, the natural history and histological characteristics of the tumors he described furnished additional tangible evidence of their right to be considered as belonging to a distinct entity.

Several qualities of these neoplasms may be taken as more or less characteristic. In regard to the clinical aspect of the picture it may be said that the tumors are relatively slow growing and that the duration of life usually is measured in years rather than months after the first appearance of the growth. Cranial metastases seem a common feature, if one may generalize on such a small series. Finally, the tumors appear to be quite sensitive to irradiation.

Examination of the specimens in gross showed them to be soft, yellow or reddish tumors, replacing bone but sharply demarcated at the margins. They were quite vascular and contained small hemorrhages within the substance of the tumor. In Stewart's three cases the tumors first appeared in a phalanx of the hand, in the fibula and in the femur. Sections showed the predominant cells to be large and arranged loosely or in an alveolar formation. The nuclei were relatively small, hyperchromatic and centrally or eccentrically situated. The abundant cytoplasm contained fine droplets of fat.

A review of the literature shows that no new cases have been reported since the appearance of Stewart's article. Bartlett ² has reported two cases of periosteal lipoma, but these did not appear to

* Received for publication July 5, 1933.

involve the bone, though one of them had to be separated from the bone by "sharp dissection." It is noteworthy that the first surgeon dealing with one of Stewart's cases removed a tumor that he found closely adherent to the fibula. He considered it as closely applied to but not arising from the bone. However, the brief descriptions of the sections do not appear to identify the tumors with liposarcoma.

Dr. Bowman Crowell of the American College of Surgeons Registry of Bone Sarcoma has kindly forwarded records and preparations of two other cases provisionally classified as liposarcoma. Concerning one of these, No. 1383, there seems to be sufficient divergence of opinion to preclude its consideration here.

The other, No. 1224,* seems to belong more definitely to this classification. The patient was a white male, 60 years of age, who was admitted to the Rhode Island Hospital in a moribund condition and who died shortly afterward. Owing to his condition biopsy or operation was not performed. The only history available was that of loss of weight and pain in the right lower quadrant for a period of one year.

At autopsy two rounded, encapsulated tumor masses about 12 cm. in diameter were found extending from each side of the ala of the right ilium. The isthmus between these, about 6 cm. in thickness, destroyed the intervening ilium; there was also some invasion of the sacrum. The surface of the tumor appeared soft and yellowish. Hemorrhagic and necrotic areas were found on section. A careful search for metastases was unavailing.

Microscopically this tumor has a good deal in common with the one reported in this paper. The cell type is quite similar. The nuclei are often hyperchromatic, usually eccentrically placed, the cytoplasm abundant and finely granular. Fat stains (sharlach R) show considerable extracellular fat, with the cytoplasm of occasional cells taking the dye. There is more evidence in favor of neoplastic origin than in our case. The alveolar arrangement is poorly developed, though present in some areas, mitoses are frequent, there is considerable variation in the size and shape of the cells, and the bizarre forms are not uncommon.

In reporting the following case we feel the same reluctance that affected Stewart in regard to making a too positive diagnosis. Although it seems justified and is corroborated by eminently compe-

* This case was reported to the Registry by Dr. B. Earl Clark.

tent pathologists the report may be met with equally competent skepticism. Nevertheless, it seems worth while to record the tumor under the present heading, and to deal with the question of reclassification when it arises.

Some of the features of the cases reported, as well as our case, are given in the table.

TABLE I

Data on Cases of Liposarcoma of Bone

Case	Age	Sex	Primary site	Cranial metastases	Duration of illness	Subsequent course
I (Stewart)	yrs. 23	M	Phalanx hand	None	yrs. 4	Living 9 yrs. after admission
II (Stewart)	28	M	Fibula	Present	1	Died 5 yrs. after admission
III (Stewart)	28	M	Not found	Present	Unknown	Died soon after admission. Disease far advanced
IV (No. 1224)	60	M	Ilium	None	1	Died soon after admission. Disease far advanced
V (S. M. H. 48952)	23	F	? Fibula	Present	5	Living 22 months after admission

REPORT OF CASE *

Clinical History: Martha A. (S. M. H. 48952), a married, white female, 23 years of age, was admitted to the Strong Memorial Hospital June 25, 1931.

Five years before admission, in 1926, the patient had undergone an operation for extirpation of a tumor of the lateral aspect of the right lower leg. Preparations of the tumor were sent to Dr. F. B. Mallory for examination. One observer, upon examining sections of the tumor returned a diagnosis of "alveolar carcinoma." Dr. Mallory states that his original opinion was that the tumor was a metastatic hypernephroma. Following operation the leg was treated once with radium. It should be made clear that we do not have any record that the tumor was attached to the fibula or its periosteum, though it must have been in close proximity.

The patient entered the hospital because of headaches that had begun six months previously. Her history was characteristic of increasing intracranial tension. Beginning with headache, the illness was successively complicated by progressive loss of vision, pain in both shoulders and paresthesias affecting both

* I am indebted to Dr. W. P. VanWagenen for permission to report this case.

hands. Three months before admission dizziness and left-sided tinnitus appeared, and the illness culminated, a few days before admission, in the development of projectile vomiting. No loss of weight was reported, and a review of the cardiorespiratory, gastro-intestinal and genito-urinary systems failed to disclose further symptomatology. The family, marital, and past history contributed nothing of interest.

Physical examination showed the patient to be well developed and well nourished. The lungs and heart were considered normal. Careful examination of the abdomen and back in the region overlying the kidneys failed to show evidence of a neoplasm. The routine neurological examination was entirely negative. Ophthalmoscopic examination, however, showed bilateral choking of the nerve heads of approximately six diopters, secondary optic atrophy, marked venous engorgement and small, scattered hemorrhages. The visual fields were concentrically constricted and exhibited a left temporal defect that was thought to be due to dilatation of a retinal vein.

The patient's hemoglobin level, white and red cell counts and urinary findings were within normal limits. Blood Wasserman test was negative. Roentgenograms of the chest and skull showed no metastases.

Two days after admission a ventriculogram was carried out which showed a slight shift of both ventricles toward the right and a deformity of the left anterior horn that suggested a mass encroaching upon it from above. At the same session a left frontal bone flap was turned down. The exposed dura was tense and thinned, but the tumor was not evident upon the surface of the cortex, nor was the surgeon able to strike either tumor or cyst with the exploring brain needle. The operation was terminated at this time because of the patient's unsatisfactory condition, but she was left with an ample decompression, and other measures were instituted to reduce intracranial tension.

Twelve days later, July 9, 1931, the left frontal bone flap was re-elevated and an incision was made in the second left frontal convolution. At a depth of approximately 4 cm. the tumor was exposed and it proved to be a discrete, soft, reddish gray mass, roughly elliptical in shape and approximately 4 cm. long. It was removed without great difficulty.

The patient's postoperative course was quite satisfactory; vision improved rapidly, and the motor aphasia that had appeared following the first operation gradually cleared up. The patient left the hospital July 31, 1931, twenty-two days following the operation.

As this paper is submitted the patient is living, but is suffering from intracranial recurrences. Relief seems to have been afforded, at least temporarily, by X-ray therapy, since these recurrences gave evidence of their existence nine months ago.

Gross Description of Tumor: The pathological report on the formalin-fixed material reads as follows: "The specimen consists of a rounded mass of white tissue measuring 2 by 3 by 3.5 cm., which is enclosed in a delicate fibrous capsule. There are many small blood vessels on the surface, which is of irregular contour owing to the projection of numerous small, rounded nodules. The tumor is of a rubbery consistence and cuts easily to present a smooth white sur-

face, dotted by a number of small, irregularly shaped hemorrhagic areas."

Microscopic Examination: Microscopic sections show vascular neoplastic tissue. The tumor cells are arranged in closely approximated small rounded groups and columns incompletely separated from one another by narrow strands of small flattened cells. There are several clear spaces of moderate size and cystic appearance which contain blood cells; in the majority of these, endothelial lining cells are not observed.

The tumor cells are large and round or polyhedral, with distinct cell membranes and abundant granular cytoplasm showing variation in depth of staining in different cells. An occasional cell contains in its cytoplasm a rounded, clear vacuole. The nuclei are large, round or oval, pale and vesicular with prominent nucleoli. The situation of the nuclei tends to be eccentric, and they lie frequently at the extreme edge of the cytoplasm. Mitotic figures are not numerous. No groups of adult fat cells are present.

Sections of formalin-fixed tissue stained for fat show moderate numbers of small red granules in some of the cells, while in the majority, deposits of fat which stain with Sudan III are not demonstrable.

DISCUSSION

The tumor was felt to be a metastatic liposarcoma. Sections were seen subsequently by Dr. F. B. Mallory, an excerpt from whose communication regarding them follows: "The best diagnosis is liposarcoma, metastatic from tumor of leg. . . . None of the cells differentiate into adult fat cells but they have the granular cytoplasm, eccentric nuclei, and alveolar arrangement which are considered characteristic." Dr. F. W. Stewart also concurred in the diagnosis, and the consensus among Drs. James Ewing, S. B. Wolbach, and J. J. Morton, who saw sections of the tumor, was that the diagnosis of liposarcoma was justified. Dr. G. H. Whipple considered the possibility of an endothelial tumor arising from blood vessels as likely.

When sections from a block of the original tumor of the leg were compared with the intracranial tumor it was Dr. Mallory's opinion that the two tumors were of identical structure. Our observations bear out this opinion. The five-year period intervening between the

excision of the tumor of the leg and the intracranial metastases argues strongly against the validity of the early diagnoses of hypernephroma and alveolar carcinoma. It is of interest that one of Stewart's cases was first diagnosed "alveolar sarcoma."

Photomicrographs of sections are reproduced to show the microscopic anatomy of the tumors, and the apparent identity of the original neoplasm with the intracranial tumor.*

SUMMARY

A case of liposarcoma with late intracranial metastases is reported. The few cases available for study seem to indicate that these tumors grow slowly, are radiosensitive, metastasize late and show a tendency to metastasize to the cranium.

The author wishes to thank Dr. F. B. Mallory for permission to use his preparations of the original tumor and for his comments on the case.

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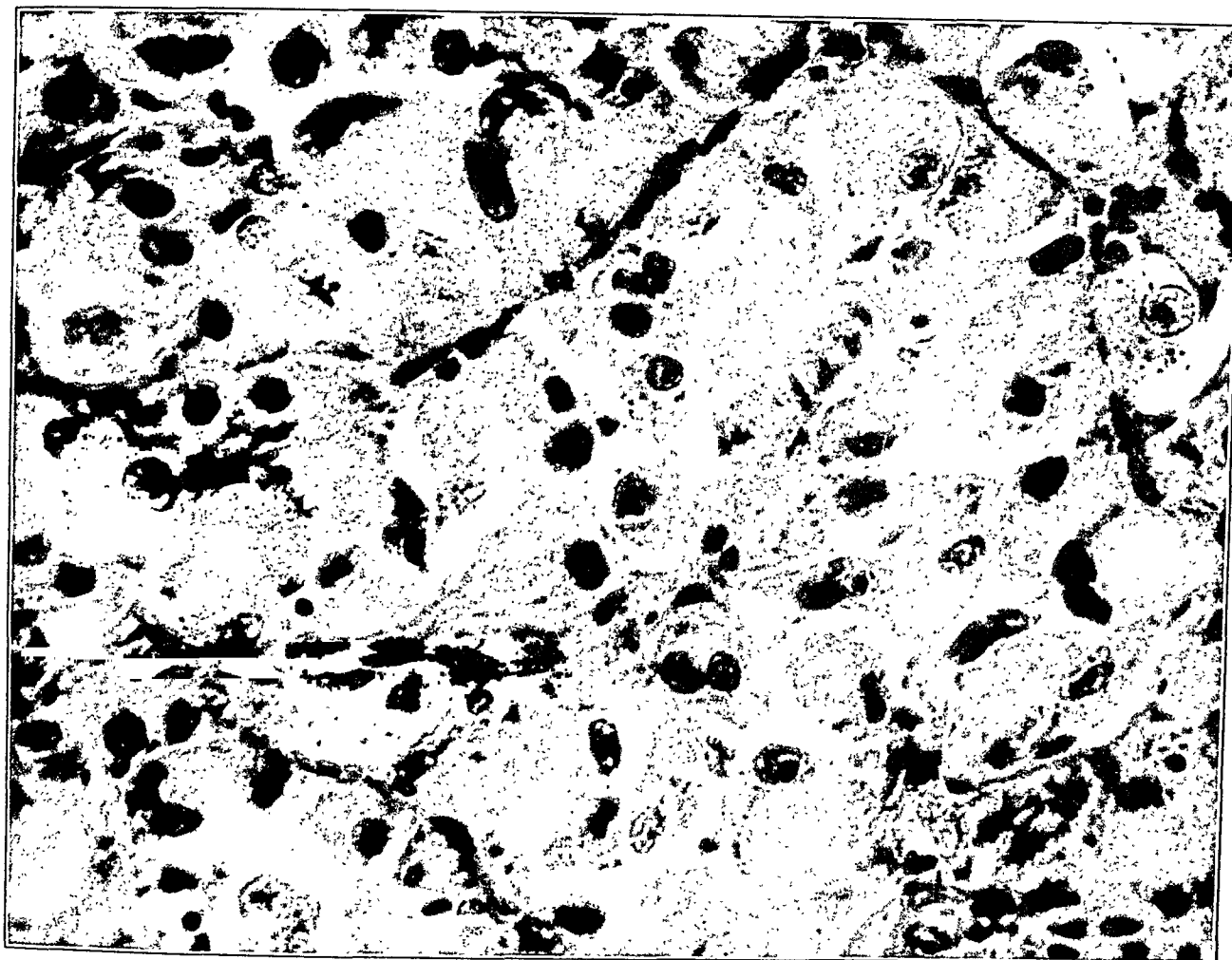
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DESCRIPTION OF PLATE

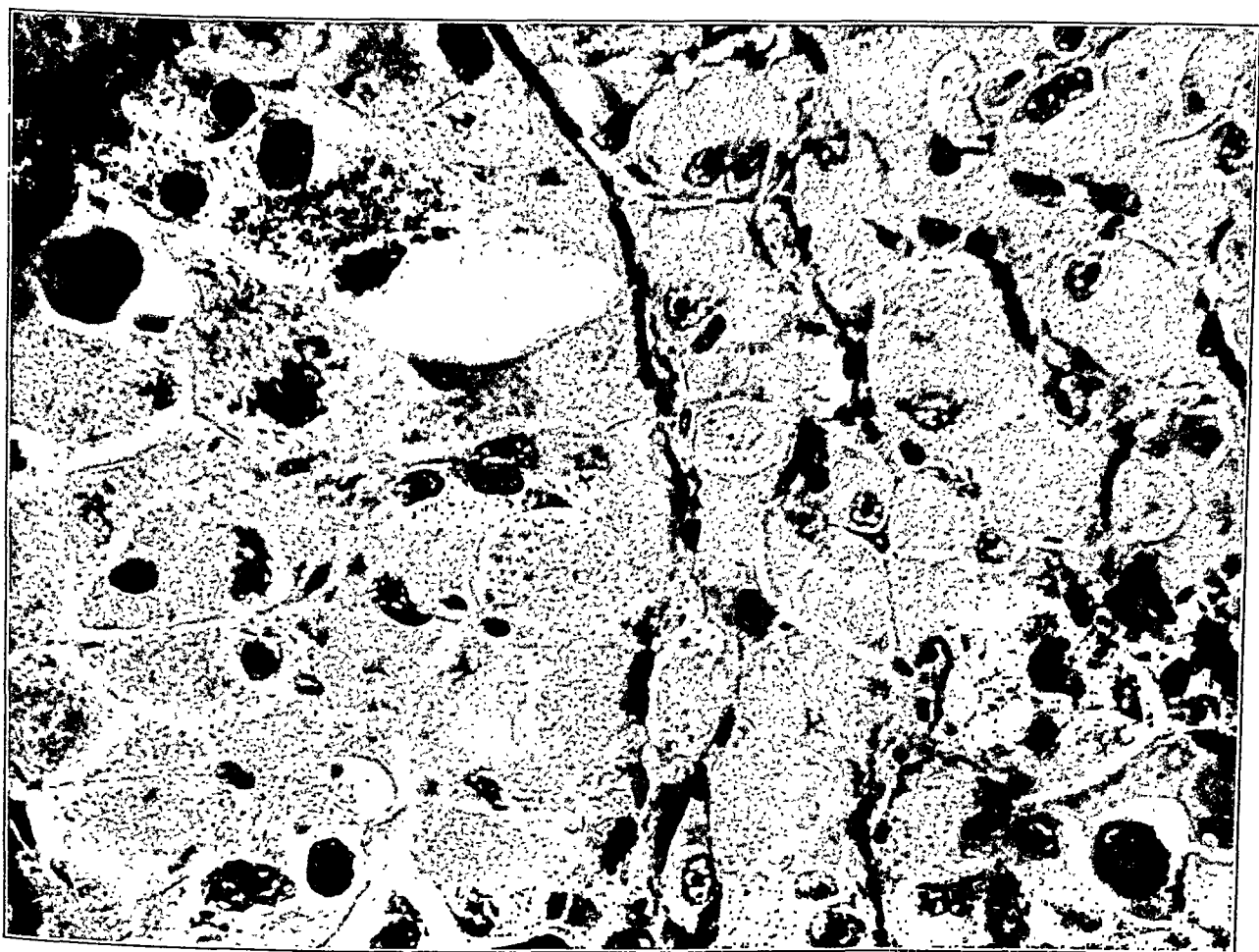
PLATE 145

FIG. 1. Photomicrograph of the primary tumor of the leg showing a rapidly growing liposarcoma of embryonic type. One mitotic figure is present. $\times 500$.

FIG. 2. A corresponding picture from the intracranial tumor, showing a similar type of new growth, evidently a metastasis. $\times 500$.



I



2

THE pH OF FORMALIN — A FACTOR IN FIXATION *
ADJUSTMENT AND STABILIZATION OF THE HYDROGEN ION
CONCENTRATION OF FORMALIN SOLUTIONS

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The appreciation of acidity and alkalinity as factors in biological staining is almost as old as staining itself. Goeppert and Cohn,¹ in 1849, made the first attempt at differentiating tissues in plants by staining with carmine. The addition of ammonia to carminic acid by Hartig,² in 1854, was found valuable in differentiating the nuclei in plant cells. Ammonia was likewise employed by Gerlach,³ in 1858. Thiersch,⁴ in 1865, observed that carmine with oxalic acid produced an orange stain and with ammonia a violet stain.

It was F. Blum,⁵ in 1893, who first employed formalin solutions for hardening and preserving tissue for histological technique. He obtained his best results with 10 per cent formalin (1 part of commercial formalin to 9 parts of water).

Soon after the introduction of formalin as a fixative, attempts were made to neutralize the solution. G. Mann,⁶ in 1894, added either sodium or magnesium carbonate to formalin with the hope of neutralizing the acid content. Up to the present time many reagents have been employed to correct the acidity of formalin. The following are those most commonly used: sodium carbonate, magnesium carbonate, calcium carbonate, lithium carbonate, magnesium oxide, ammonia and sodium hydroxide. Atkins,⁷ in 1922, suggested the use of borax. It occurred to the author that pyridine, because of its chemical properties, would be a more suitable reagent.

In the preparation of so-called neutral formalin the fundamental concept of base neutralizing acid has been in the minds of several masters of histological technique. However, in preparing such a formalin solution it is essential to consider the nature of formaldehyde, as well as that of the reagent employed. All dilutions of commercial formaldehyde in distilled water are acid. In addition, there are present several factors that tend toward acidification: (a) pro-

* Received for publication June 27, 1933.

duction of formic acid upon the oxidation of formaldehyde; (b) absorption of carbon dioxide from the air; and (c) liberation of acid from the tissue immersed in the solution. Furthermore, commercial formalin usually contains methyl formate, the saponification of which would also tend to depress the pH of solutions to which base is added. When base is added to an aqueous solution of formaldehyde an increase in pH results. However, a decrease in pH follows.

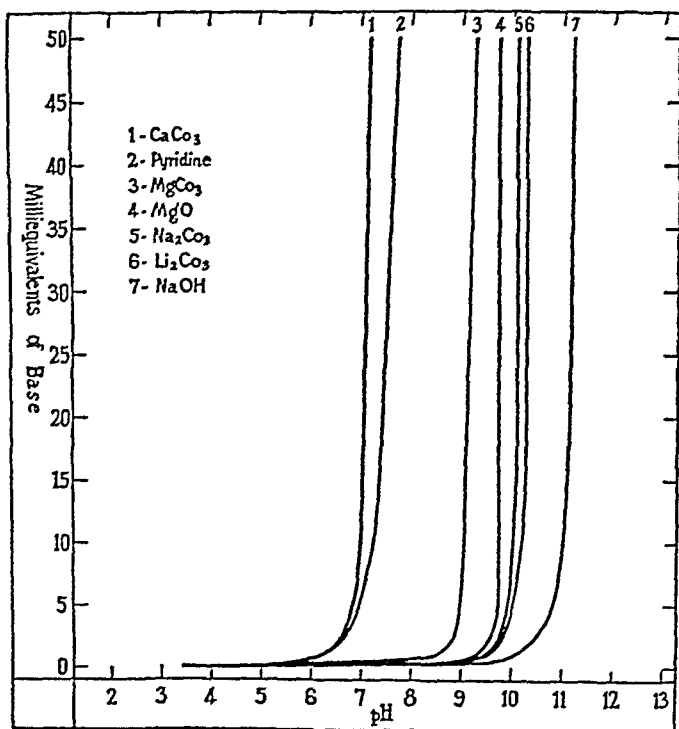


CHART I

This lowering of the pH cannot be entirely checked, but the speed at which acidification becomes manifest can be affected by the addition of an excess of the reagent employed. The addition of an excess of base, if approximate neutrality be desired, necessitates a consideration of the maximum buffering range* that will obtain when the selected reagent is dissociated in an aqueous solution of formaldehyde. It is this that determines the range of maximum stability of the pH of the fixing fluid.

Chart 1 presents a comparison of the maximum buffering ranges that obtain upon the addition of the several reagents in common use

* By buffer action is meant the resistance to change of pH exhibited by a solution when it is subjected to gain or loss of acid or alkali, according to Clark.⁸ The pH range at which this resistance to change takes place is known as the maximum buffering range.

to 25 per cent commercial formalin in distilled water. Each pH determination was made on individual samples of 100 cc. of 25 per cent commercial formalin in distilled water, to each of which was added a definite quantity of one of the several reagents employed. The pH determinations were made by the Glass Electrode within one hour after the addition of each reagent. It is obvious that with the exception of calcium carbonate the common reagents buffer best at pH values well above neutrality. This means that such a concentration of base as will produce neutrality will not produce a stable solution. In the same figure we find also that pyridine or calcium carbonate might be added in excess without causing a marked rise in pH. Conversely, an excess having been added, consumption of either pyridine or calcium carbonate is accompanied by a very slight decrease in pH. It is this feature of the latter that makes them the reagents of choice when relatively stable neutral formalin is desired.

The author has selected pyridine, instead of calcium carbonate, for several reasons. Pyridine-formalin is a relatively stable solution without a precipitate and void of all alkali earth metals, which appear to be objectionable elements in subsequent silver staining. Ammonia will not serve the same purpose as pyridine because it reacts with formaldehyde to form the very weakly basic condensation product hexamethylenetetramine. Formalin solutions that have been adjusted to pH values in the alkaline range by addition of ammonia, therefore, regain part of their acidity on standing, the pH level sinking below pH 7.0. Pyridine, being a tertiary base, is unable to undergo any such condensation with formaldehyde. In addition, the properties of pyridine itself are most desirable in fixation. Pyridine has long been recognized as a hardening, dehydrating and clearing agent. Furthermore, the formation of paraformaldehyde, an undesirable precipitate, seems to be checked if not completely avoided in pyridine-formalin.

Chart 2 indicates the effect of pyridine on the pH of 25 per cent commercial formalin to which it is added. I have found that pyridine-formalin made up as follows is most satisfactory for fixation of tissue:

Distilled water	75 cc.
Commercial formalin	25 cc.
Pure pyridine	5 cc.

Tissue should be immersed in approximately 20 times its weight of pyridine-formalin. Forty-eight hours is sufficient time for fixation. In preparing tissue for peripheral nerve stains 2 weeks is preferable. No injury to tissue has been noticed after 6 months immersion in pyridine-formalin, and the formation of formalin pigment has been prevented. Tissues so fixed are suitable for all the usual

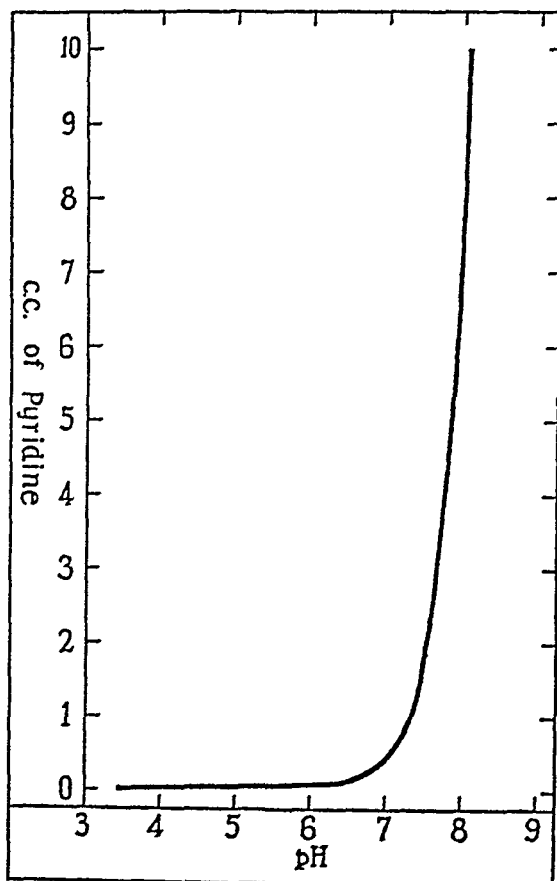


CHART 2

stains, such as hematoxylin and eosin, the Masson-Mallory trichrome, fat stains, the Laidlaw collagen-reticulin stain, the dopa reaction, and so on. Pyridine-formalin has proved to be far superior to all other fixing fluids in preparing tissue for the Laidlaw modification of the Gros-Bielschowsky silver stain for neurites in frozen sections.⁹

Embryonic bone and the bone that is formed in tissue culture are susceptible to even a low degree of acidity in the fixing fluid. Hence alcohol or neutral formalin are the only available fixatives for this material. And, since in work with experimentally produced bone it

is desirable to avoid the presence of the alkali earth metals in the fixing fluid, pyridine-formalin affords a convenient and easy solution of the formalin problem (Murray¹⁰). In tissue culture fixation a 5 per cent pyridine-formalin solution is used. This may be prepared by diluting 1 part of the standard 25 per cent pyridine-formalin solution with 4 parts of either distilled water or normal saline solution.

The following table presents a series of determinations made on routine surgical specimens fixed in 25 per cent pyridine-formalin.

TABLE I

Serial No.	Laboratory No.	Surgical specimen	Fluid-specimen ratio	Time	pH	pH change
				<i>days</i>		
1	51726	Hernial sac	28.5 -1	9	7.62	-0.19
2	51730	Thyroid	2.5 -1	8	7.04	-0.77
3	51739	Testicle and hernial sac	1.9 -1	6	6.81	-1.00
4	51748	Breast	1.7 -1	5	7.00	-0.81
5	51754	Hemorrhoids	21.2 -1	4	7.63	-0.18
6	51755	Hemorrhoids	25.0 -1	4	7.63	-0.18
7	51765	Abscess right leg	12.5 -1	3	7.32	-0.49
8	51768	Thyroid	1.8 -1	1	6.99	-0.82
9	51770	Cervix	3.6 -1	7	7.08	-0.74
10	51771	Colostomy	7.2 -1	1	7.50	-0.31
11	51778	Tumor bronchial region	12.5 -1	7	7.55	-0.27
12	51784	Dermoid cyst	16.6 -1	6	7.55	-0.27
13	51796	Thyroid	1.5 -1	5	6.99	-0.83
14	51805	Thyroid	5.4 -1	4	7.16	-0.66
15	51806	Thyroid	1.1 -1	4	6.85	-0.97
16	51812	Cervical lymph nodes	11.7 -1	3	7.44	-0.38
17	51821	Curettings from femur	12.4 -1	3	7.59	-0.23
18	51827	Hemorrhoids	19.5 -1	2	7.65	-0.17
19	51831	Appendix	4.4 -1	8	7.16	-0.66
20	51832	Appendix	7.3 -1	8	7.44	-0.38
21	51833	Hemorrhoids	17.4 -1	8	7.59	-0.23

In Table I we find that only a slight change in pH takes place over a variable period of time. After tissue has been fixed (24 to 48 hours) the pH drop is practically checked. It is obvious that the fluid-specimen ratio (cc. of fluid to gm. of tissue) is an important factor. If the recommended 20 to 1 ratio be followed, the drop in pH will usually be not more than 0.3 of a pH unit over a period of fixation.

Finally, pyridine-formalin is the fixative of choice whenever neutral formalin is desired, and since such formalin is optimum

for fixation, pyridine-formalin for fixation of tissues in general is recommended.

NOTE: It is a pleasure to thank those whose advice and coöperation have been valuable factors in preparing this paper, among them Prof. Arthur Purdy Stout, director of the laboratory, and Dr. Margaret R. Murray of the department of surgery, Profs. H. T. Clarke and E. G. Miller, and Mr. F. Rosebury of the department of biochemistry. The writer is especially indebted to Dr. George F. Laidlaw of this laboratory who has given generously of his time and of his rich experience in histological technique.

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SCIENTIFIC PROCEEDINGS OF THE
THIRTY-THIRD ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS AND
BACTERIOLOGISTS

WASHINGTON, D. C.

May 9 and 10, 1933

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

The following officers were elected at the meeting May 9, 1933:

<i>President</i>	O. T. AVERY
<i>Vice-President</i>	WILLIAM BOYD
<i>Treasurer</i>	F. B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	E. R. LONG
<i>Assistant Secretary</i>	ROBERT A. MOORE

ABSTRACT OF MEETING OF THE COUNCIL

Voted to elect the following new members:

John T. Bauer	Frederick C. Narr
Thomas H. Belt	James M. Neill
Robert M. Choisser	James L. O'Leary
Herman R. Churchill	Frank B. Queen
Robert S. Cunningham	Herbert L. Ratcliffe
William A. DeMonbreun	Harry L. Reinhart
Vera B. Dolgopol	Arnold R. Rich
Robert C. Grauer	Thomas M. Rivers
Eleanor M. Humphreys	Florence R. Sabin
Raymond A. Kelser	Grace M. Sickles
Eric A. Linell	John W. Spies
Ralph E. Miller	Harold L. Stewart

Voted to accept with regret the resignations of Drs. H. L. Amoss, A. J. Chesley, Stanley Cobb, S. J. Crowe, I. C. Hall, A. T. Hess, J. A. Kolmer, S. H. McKee, R. G. Mills, A. G. Nichols, T. P. Sprunt, Ruth Tunnicliff, G. H. Weaver, Toynbee Wight, and R. T. Woodyatt.

Voted to elect Frederic Parker, Jr. as Assistant Editor of the *American Journal of Pathology*.

Voted to authorize the Editorial Board to issue a special number of the *American Journal of Pathology* in honor of Dr. Frank B. Mallory.

Voted to appoint Drs. Ewing, Callender and Mallory a committee on the publication of the report of the Registry of Lymphatic Tumors, with power to act.

Voted to select as a symposium topic for 1934 the subject of arteriosclerosis and to request Dr. Oskar Klotz to serve as referee.

The Secretary was directed to place in letters of acceptance of resignations a statement that the Council would gladly consider reinstatement at any time the person sees fit to notify him that he wishes reinstatement.

Voted to adopt the following resolution:

The American Association of Pathologists and Bacteriologists expresses entire sympathy with the Medical Library Association in its protest against the cost of German medical periodicals and will, in so far as possible, engage to support any means that may be adopted to enforce action by the German publishers.

Voted to accept the invitation of the University of Toronto and to meet in Toronto March 29 and 30, 1934.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A UNIQUE INFECTION IN MAN WITH A NEW YEAST-LIKE ORGANISM. G. H. Hansmann and (by invitation) J. R. Schenken, Iowa City, Iowa.

Abstract. A white man, 43 years of age at the time of death, had had an irtractable skin affection during the last 16 years of his life. His residence during this entire period was in Iowa. The first lesions were noted in 1916 on the skin in back of the knees. The extension of the disease was gradual and by 1929 the entire skin was involved by a scaly dermatitis, underneath which the skin was somewhat thickened and reddened. The peripheral lymph nodes became enlarged, the larger nodes measuring 3 cm. in diameter. During 1929 the skin became more reddened and thickened and cracked easily. In June, 1932, the lesions became papular in character, so that by July the skin was thickly set with hard, umbilicated papules that measured 0.5 cm. in diameter. He developed a high fever late in July, which was associated with evidence of pleurisy and a systemic infection. Death occurred Aug. 7, 1932.

A papule and a lymph node were removed during life for study. Both contained an organism that appeared as a small yeast. It was situated largely within large mononuclear leukocytes and giant cells. There were no intra-epithelial abscesses. The organism measured 5 microns, while the yeast-like cell proper measured 3 microns. Postmortem examination revealed localization of the organism in the lungs and adrenals, in addition to the skin and lymph nodes.

The organism had no particular food requirements. The appearance, after 6 days, of flat, icy-appearing, filamentous specks on the surface of the medium was characteristic of the early colonies. The medium was not deeply invaded. Sugars were not fermented. The characteristic finding was numerous, large, spiculated chlamydospores. They appeared on lateral branches near the center of the colony.

The organism was pathogenic for guinea pigs, rabbits, rats and dogs. The dog was particularly susceptible. Generalized lesions were seen in this animal 4 weeks after an intravenous inoculation. These lesions were largest in the lung and spleen. The yeast-like organism was isolated from the various organs.

The spiculated chlamydospore is distinctive. The yeast-like organism in tissues is smaller than any described heretofore. The character and distribution of lesions in man are unlike those of any other disease. The organism is a member of the genus *Sepedonium*. So far as we are able to tell, it is the first pathogenic member of this group.

THE PATHOLOGICAL ANATOMY OF ACTINOBACILLOSIS IN MAN AND EXPERIMENTAL ANIMALS. D. C. Beaver, Rochester, Minn.

Abstract. Previous instances of human infection by the *Actinobacillus*, namely, the case of meningitis reported by Rivout and Pinoy, and the case of *Actinobacillus* bacteremia reported by Thompson and Willius, afforded no opportunity for pathological investigations, since these cases terminated in recovery. This, the third human case to be reported, is the first to result in death and therefore the first upon which pathological study has been made.

Although the exact nature of the lesions of human actinobacillosis has been unknown, their characteristics might have been anticipated from a study of the lesions in cattle, where the disease frequently occurs spontaneously. Bovine lesions are essentially chronic granulomatous abscesses, in the exudate of which pseudosulphur bodies are found. The sulphur granules of bovine actinobacillosis are composed of closely set, small Gram-negative bacilli in ball-like colony formation, surrounded by radiating pseudoclubs. They are never composed of entangled masses of Gram-positive mycelia with peripheral radiating clubs, as in actinomycosis.

The human lesions, as exemplified by this case, were multiple, discrete, granulomatous abscesses and involved the liver, spleen and lungs. The spleen was most severely affected and weighed 748 gm. The abscesses were composed either of thick creamy pus, or more frequently of material of thick pasty consistence, resembling caseation necrosis. Microscopically the lesions were focal and sharply circumscribed. The central portions were usually composed of granular, acellular, eosin-staining material in which fragmented nuclear remnants were prominent. Polymorphonuclear leukocytes were abundant in a zone peripheral to the central necrosis. An outermost zone was composed predominantly of endothelial cells and fibroblasts. The lesions were avascular. Masses of bacteria in ball-like colony formation were frequently associated with the layer of polymorphonuclear leukocytes. They resembled the central portion of the "sulphur granules" as found in bovine actinobacillosis, but peripheral clubs were never found in the human tissues.

The organisms, as recovered from the human lesions, and from the ante mortem blood culture, were proved to be pathogenic for rabbits, guinea pigs and mice. Granulomatous abscesses and tubercle-like granulomata were formed. In the lesions of experimental animals the granulomatous features were frequently much more prominent than they were in human lesions; nevertheless, they were similar to if not identical with one another. Bacterial masses, in ball-like colony formation, were found in many of the infectious foci of the experimental lesions. Peripheral clubs were never present, as in bovine actinobacillosis, although suggestions of early pseudoclub formations were sometimes observed.

ACTINOMYCOSIS OF TUBES AND OVARIES. V. H. Cornell, Washington, D. C.

Abstract. The literature of actinomycosis, in which cases of the internal female genitalia were involved, has been reviewed to date. There have been 66 cases reported, the author's bringing the total to 67. The longest previously reported cure was 3 years and 1 month; the individual in the case here reported has now been entirely well for 4 years.

The case presented was that of a white woman, 31 years of age, who had had pain in the right lower quadrant since 1919. Appendectomy was performed Aug. 10, 1924, but no histological record is available. Pain recurred and continued until acute illness caused admission to the hospital May 3, 1929. Partial intestinal obstruction and a mass in the right pelvis were the preoperative diagnoses. Adhesions were broken and a right tubo-ovarian mass was removed. Colonies of actinomyces in an enlarged ovary and an attached tubal mass were found histologically in the midst of multiple communicating abscesses, all enmeshed in dense fibrous tissue. No cultures were made as the nature of the condition was not recognized until sections were studied.

Discussion

(Dr. Esmond R. Long, Chicago.) It was not clear to me what was the source of the infection. How did it arise in this location?

(Dr. Cornell.) I presume that it was possibly appendiceal in origin. I tried to get the appendix, which had been previously removed, but no sections had been made and the gross specimen had been discarded.

(Dr. Long.) That would be the most likely source.

(Dr. Cornell.) It seems to me to be the most likely.

TUBERCULOUS ENDOPHLEBITIS WITH OBLITERATION OF THE SUPERIOR VENA CAVA. REPORT OF A CASE. H. C. Schmeisser, Memphis, Tenn., and (by invitation) Henry Fuller, Mulberry, Fla., and I. H. Jones, Paris, Tenn.

Abstract. This condition is not only very interesting but extremely rare. We have found only one case in the literature, that reported by Prof. Guido Banti in 1891. His case was a male, aged 46 years, a native of Florence, who at autopsy showed the superior vena cava to be converted into a hard cylinder with a vegetative mass occupying its auricular entrance, which microscopically and bacteriologically proved to be a tuberculous endophlebitis. No clinical record of importance was obtained.

Our case occurred in a female, aged 24 years, a negress. The clinical record was complete. The heart at autopsy showed the lower part of the left internal jugular, both innominates, except for a small superior part of the right, and the entire superior vena cava to be completely obliterated by a tuberculous endophlebitis without thrombosis. The auricular orifice of the cava was completely covered by endocardium. Tubercle bacilli were demonstrated in sections of the intimal lesion. The enlarged right innominate vein pressed on the recurrent laryngeal nerve and the cava slightly compressed the right bronchus. The left common carotid artery took origin from the innominate artery 0.5 cm. above the origin of the latter from the arch of the aorta. The inferior vena cava was dilated.

The obstruction involved the entrance of the right and left inferior thyroid and internal mammary, left vertebral, subclavian and highest intercostal, and azygos veins. It was necessary for the blood from the head, neck, arms and thorax to return to the heart by means of a collateral circulation and the inferior vena cava.

The entire systemic venous circulation, except that of the heart, was in a state of chronic passive congestion and many normally insignificant veins had become prominent. This explained such symptoms as dyspnea, orthopnea, cyanosis and dropsy. Hoarseness and cough were due to pressure of the enlarged right innominate vein on the right recurrent laryngeal nerve.

The anomaly of the left carotid artery taking origin from the innominate artery might suggest that the closure of the auricular orifice of the superior vena cava was congenital. Could the patient have had her abnormal circulation for 24 years? After a congenital closure, tubercle bacilli from the focus in the lung or pleural cavity could have entered the circulation and could have settled out from the blood into the superior vena cava, causing the endophlebitis. This might explain the occurrence of a tuberculous endophlebitis of the superior vena cava, which must be considered very rare.

CULTIVATION OF ACID-FAST BACTERIA IN CHICK EMBRYO. Elizabeth Verder
(by invitation) and Earl B. McKinley, Washington, D. C.

Abstract. Data are presented on the cultivation of several acid-fast bacteria in minced chick embryo in Tyrodes's solution, with and without the addition of 3 per cent glycerol. Comparisons are made with several of the cultures employed with cultivation on the ordinary laboratory media usually used for acid-fast organisms. The organisms studied include fresh human strains of *B. tuberculosis*, a bovine strain of *B. tuberculosis*, a freshly isolated strain of avian *B. tuberculosis*, strains R1 and H37 of *B. tuberculosis*, 4 strains of *B. leprae* recently isolated by us from fresh leprosy nodules, and the Duval and Kedrowski strains of so-called *B. leprae*. Data are also given on the cultivation of several of these strains on the oyster-egg medium recently described by us. In general, with the various strains of *B. tuberculosis* where growth is obtained on the usual laboratory media used for acid-fast organisms, growth is also obtained in minced chick embryo tissue medium. However, in many instances growth in the tissue medium is more rapid and appears as early as 10 days following inoculation. The freshly isolated strains of *B. leprae* commonly require at least 1 week for incubation, or as long as 10 to 12 days. Growth is definite, but by no means luxuriant, the same being true for the human strains and bovine strains of *B. tuberculosis*. The avian strain of *B. tuberculosis* and the Duval and Kedrowski strains of so-called *B. leprae*, however, grow very luxuriantly in the tissue medium. The addition of glycerol to the tissue medium favors growth of our strains of *B. leprae*, but apparently does not enhance conditions for multiplication in tissue medium for the human and bovine strains of *B. tuberculosis*. Details of the method used for cultivation in chick embryo tissue medium are fully described.

Discussion

(Dr. Esmond R. Long, Chicago.) This has always seemed to me to be the most logical method to use in the cultivation of pathogenic organisms. It is a pretty rude jolt to bacteria to take them from living tissues and put them in the artificial media which we use. Moreover, in recent years we have learned that we can modify the morphology and possibly also the virulence of microorganisms by growth on different artificial media, so that it would be the most logical thing to put them in tissues where they are most likely to retain their pathogenic property. I was interested to hear that Dr. McKinley is using human tissues for his growth of acid-fast bacilli. It seems to me that is carrying logic to the most desired point.

EXPERIMENTAL EPIDEMIOLOGY OF TUBERCULOSIS. THE EFFECT OF A PRIMARY
INFECTION WITH TUBERCLE BACILLI OF THE HUMAN TYPE ON CONTACT
TUBERCULOSIS IN RABBITS. Max B. Lurie, Philadelphia, Pa.

Abstract. Normal rabbits exposed to tuberculous cage mates infected with the bovine bacillus acquire a respiratory or an alimentary infection that is fatal in 50 per cent of the cases. The incidence of this naturally acquired tuberculosis was 73 per cent within a year of exposure.

A large proportion of rabbits vaccinated with virulent tubercle bacilli of the human type are completely protected from natural infection for the first 6 months of the exposure. This protection wanes with further exposure and some rabbits succumb to an exogenous, naturally acquired reinfection. Both the

human tubercle bacillus of the primary infection and the bovine bacillus of reinfection may be isolated in pure culture from the same rabbit.

The vaccination reduces the incidence and mortality of the disease, changes its pathological characteristics, and retards its progress.

A method is offered whereby naturally acquired tuberculosis closely simulating the natural infection in man can be studied in a small laboratory animal.

Discussion

(Dr. E. T. Bell, Minneapolis.) This work appears to demonstrate some protection against tuberculosis of the contact type.

(Dr. Joseph D. Aronson, Philadelphia.) It appears to me that the most interesting group of animals in Dr. Lurie's experiments are those among the vaccinated rabbits which survived for a long period of time and among whom there was a high mortality from tuberculosis. Is it possible that in these vaccinated animals the tubercle bacilli are to a great extent destroyed, leaving a small number of virulent tubercle bacilli which multiply slowly, or that the virulence of the organisms increases so as to overcome the extent of vaccination?

I should like to ask Dr. Lurie whether the cultures of tubercle bacilli isolated from vaccinated rabbits show any difference in degree of virulence, as compared to cultures of tubercle bacilli isolated from the unvaccinated ones.

(Dr. Esmond R. Long, Chicago.) One important point that Dr. Lurie made clear is that in spite of vaccination, in the long run, the vaccinated animals tended to develop tuberculosis. In fact, his figures at the late period showed a larger number of animals developing tuberculosis among the vaccinated than among the unvaccinated. That might be accidental, as 30 or 40 animals may not have been a large enough series to settle the point. An important matter is to determine individual variations in susceptibility in normal animals, and Dr. Lurie at the present time is studying families of rabbits which are somewhat analogous to human families, and is trying to learn if constant variations in susceptibility occur.

Another point he made, but might have stressed more, is that when such variations to susceptibility can be established, one may infer from certain observations that this increased natural resistance can be enhanced by vaccination. Vaccination is not so much of a help to animals that have an extremely low natural resistance. So we must study the factors of natural susceptibility and resistance as part of this problem.

(Dr. Lurie, closing.) In reply to Dr. Aronson's question, I have isolated tubercle bacilli from normal animals that have acquired tuberculosis, and also from vaccinated animals that have acquired tuberculosis, and I have determined their virulence and, so far, there was no difference in the virulence of the strains, whether they originated from normal animals or vaccinated animals.

I wonder if I have the right to make the following suggestion. I feel that the reason why those 7 vaccinated animals could have been deleteriously influenced by vaccination may be due to the fact of fixation of bacilli. It is well known that sensitization results in fixation of bacilli at the portal of entry, whatever the mechanism of fixation. We also know that no matter how great the immunity that results from infection, it is always relative. In view of the fact that many bacilli are constantly absorbed, especially in the intestines, it is possible that many more bacilli will accumulate in given localities in the vaccinated than in the normal animal, as a result of the tendency of fixation of bacilli by the sensi-

tized animal. In the normal animals fewer bacilli become fixed in the same localities. In the vaccinated animals the immunity is overwhelmed by the large accumulation of bacilli at the portal of entry. The smaller numbers of bacilli present at any one time at the portal of entry of the normal animals may be destroyed by the factors of inherent resistance.

In reply to Dr. Long, I have not stressed the factors of natural resistance, but it is really very important. After all, this vaccination has only decreased the mortality by 13 per cent in the long run, although in the first 6 months the protection was great, because only half as many normal animals escaped infection. The question of natural resistance in infectious diseases is now becoming more and more important.

As to the question of Dr. Bell, we only used subcutaneous inoculation because we wanted to have a lesion that would be localized as much as possible, but even in those cases where rabbits developed a generalized infection with localization in the lung and the kidney as a result of vaccination, exogenous reinfection occurred, so that I question whether if we had vaccinated them in a different way it would have materially influenced the course of the disease.

A FACTOR IN THE MECHANISM OF INVASIVENESS BY PYOGENIC BACTERIA. Valy Menkin, Boston, Mass.

Abstract. Trypan blue injected into an area of cutaneous inflammation induced by *Staphylococcus aureus* fails to drain readily to the tributary lymphatics if the dye is injected as early as 1 hour after the inoculation of the microorganisms.

Trypan blue introduced into an area of cutaneous inflammation induced by *Pneumococcus* Type I is retained *in situ* if the dye is injected about 6 or more hours after the inoculation of the bacteria.

When an area of cutaneous inflammation is induced by the inoculation of a culture of *Streptococcus hemolyticus*, trypan blue injected into it drains readily to the tributary lymphatics for the first 30 hours following the onset of the inflammatory reaction. If the inflammation has lasted for 45 hours or longer, the dye is fixed *in situ* and fails in most instances to reach readily the tributary lymphatics.

The rapidity of fixation of the dye is correlated by simultaneous mechanical obstruction in the form of a fibrinous network and thrombosed lymphatics, or of thrombosed lymphatics alone at the site of inflammation induced by each of the aforementioned pyogenic organisms.

Inasmuch as staphylococci, pneumococci, and streptococci disseminate from the site of their cutaneous inoculation primarily through lymphatic channels, the difference in the rapidity with which mechanical obstruction is set up in the respective inflamed areas is a factor in explaining the invasive abilities of these pyogenic organisms.

Discussion

(Dr. E. T. Bell, Minneapolis.) Dr. Menkin has done very interesting work on the fundamental phenomena in inflammation. He stresses, as most of you know, the mechanical factors in preventing the spread of organisms, and I gather that he thinks mechanical factors are much more important in the later than in the earlier stages of inflammation.

(Dr. Arnold Rich, Baltimore.) I should like to ask Dr. Menkin a question. As I understand it, he feels that the relative speed with which the lymphatics

become thrombosed is the chief factor that determines whether or not bacteria will spread from a site of infection. It is true that in the human being streptococci are often highly invasive, but in the rabbit it is almost impossible to find a streptococcus which, if injected into the skin, will spread readily through the tissues and invade the blood stream to produce a fatal septicemia. On the other hand, the Type I *Pneumococcus* is one of the most highly invasive bacteria imaginable in the rabbit. Injected into the skin, it invariably spreads to produce huge local lesions, and it invades the blood stream within several hours, the animal dying in a day or so with generalized septicemia. Now, according to Dr. Menkin, the streptococcus (which, in the rabbit, has very feeble invasive powers) does not cause early thrombosis of lymphatics in that animal, whereas the pneumococcus, which is extremely invasive, does do so. I should like to ask Dr. Menkin how he reconciles these facts with his view that thrombosis of lymphatics is the factor that prevents invasion.

(Dr. Menkin.) In answer to the question that has been raised by Dr. Rich, I should like to point out that when one introduces into the skin a substance, whatever the substance may be, whether it is inorganic or bacterial, a part of it disseminates promptly into the lymphatics and thence into the blood stream. Consequently when one inoculates a rabbit intracutaneously with *Pneumococcus* Type I, some of these microorganisms doubtless disseminate into the blood stream at least within the first 6 hours. In fact, we have in the first 6 hours obtained pneumococci by culturing the lymph obtained from the efferent lymphatic vessel. These organisms will ultimately reach the blood stream and produce a positive blood culture. Evidently those animals in which multiplication in the blood stream of pneumococci is more extensive probably succumb to the infection; but this has little to do with what is occurring at the site of inoculation, for although some cocci may have disseminated immediately after their inoculation, the infected skin area, as it develops an inflammatory reaction, will be gradually shut off, and the quicker it is circumscribed the fewer bacteria will disseminate eventually into the blood stream.

(Dr. Rich.) But did the streptococci invade the tissues and blood stream of your animals as freely as the pneumococci? Did your blood cultures and lymph cultures show a comparable dissemination?

(Dr. Menkin, closing.) Yes, indeed, streptococci disseminate readily into the tributary lymphatics from which they have been cultured. We are discussing dissemination and not virulence or resistance. Dr. Rich is evidently discussing the question of virulence and resistance, which is not the same as dissemination. In other words, if an organism reaches the blood stream very soon after its dermal inoculation, and if it happens to be extremely virulent for the rabbit, it will multiply rapidly in the circulation, causing ultimately death from septicemia, irrespective of the fact that the site of inoculation in the skin might at the time of death be completely walled off. The streptococci, on the other hand, disseminate freely into the blood stream, for there is apparently nothing that walls them off, and therefore retard their passage for the first 2 days after their cutaneous inoculation. However, the animal does not necessarily die if the particular strain of streptococci used happens to be avirulent for the rabbit. Resistance is not to be confused with dissemination, as has often been done in the literature.

THE HEART VALVES IN EXPERIMENTAL SCURVY AND IN SCURVY WITH SUPERIMPOSED INFECTION. James F. Rinehart and (by invitation) Stacy R. Mettier, San Francisco, Calif.

Abstract. In experiments designed primarily to study the reaction of the blood and blood-forming tissues of the scorbutic guinea pig to infection, routine examination of the heart revealed occasional peculiar proliferative lesions in the heart valves. This led to a more detailed study of the effect of scurvy and of scurvy combined with infection upon the heart valves, heart muscle and pericardium in the guinea pig. The diet used is a modification of that of Dahldorf and has proved quite adequate for growth and maintenance when supplemented with orange juice. Vitamins other than C are present in generous amounts and the mineral content is adequate. The infecting agent used was a hemolytic streptococcus derived from the spontaneous lymphadenitis common in the guinea pig (*B. aertrycke* was used in a small independent series of animals). The streptococcus was transmitted by intracutaneous inoculation of a pure broth culture. This produced a skin infection and infection of the regional lymph nodes. It is comparatively unusual for it to produce metastatic blood-borne lesions.

More than 90 animals were used in the study, divided into the following general groups:

1. Dietary controls — basal diet supplemented with adequate daily orange juice.
2. Acute scurvy — basal diet alone.
3. Subacute or chronic scurvy — basal diet with inadequate amounts of orange juice.
4. Dietary controls infected by intracutaneous inoculation of hemolytic streptococci.
5. Acute scurvy with superimposed streptococcus infection.
6. Subacute or chronic scurvy with superimposed infection.
7. Infection with superimposed scurvy (subacute).

The experiments were performed in four series. The results were sufficiently consistent in each series to justify the observations set forth.

Control animals show essentially normal heart valves. The normal valves possess a compact appearance with a rich, closely set, wavy, collagen fiber structure. A delicate endothelial surface layer is visible. Of 8 dietary control animals, adequate sections were obtained in 6. Of these, the valves showed a normally rich fiber structure in all. One animal showed a very mild proliferation of the endothelium overlying the insertion of one of the large chordae tendineae.

The animals on the basal diet supplemented with orange juice and experimentally infected with streptococci showed essentially normal valves in all but three instances. One animal showed an acute, necrotizing mitral valvulitis; the second an accumulation of a few polynuclear leukocytes near the base of the tricuspid valve, with some associated capillary endothelial hyperplasia. The third animal developed a uremia from an obstructed urinary tract and showed a peculiar collagen degeneration in the mitral valve. In a few other instances there were seen a meager increase in the free cells in the mitral valve and an occasional small questionable focus of edema or degeneration. The hearts of 15 animals were adequately examined in this group. No proliferative reactions were seen, such as occurred in the groups to be described.

In acute or severe chronic scurvy without infection, definite degenerative lesions were observed in the heart valves. The normally rich fibrous structures became impoverished. The valves showed thinning, fragmentation and disorganization of the regular axial arrangement of the fibers. A loss of the normal wavy contours and at times a hyaline degenerative change of the fiber substance was observed. Nine of ten hearts adequately examined in uncomplicated scurvy revealed changes of this sort. In two instances mild but definite proliferative reactions were noted at the contact area of the mitral valve. The nuclei of the stroma cells frequently showed shrunken pyknotic contours. In animals subjected to both scurvy and infection striking pathological changes were observed in considerable numbers. The findings were consistent in each of four series of experiments. The lesions observed were a combination of the degenerative effects described in scurvy with, in addition, in many instances, proliferative reactions on the part of the stroma and surface endothelial cells. Thirty-one animals were subjected to a combination of scurvy and streptococcus infection. Of these a reasonably adequate examination of the heart valves was secured in 24 animals. Practically all of these 24 animals presented recognizable degenerative changes in one or more heart valves. In addition 16 animals (66 per cent) showed moderate or marked proliferative reaction on the part of the stroma and surface endothelial cells in one or more of the heart valves. The proliferated cells present one or more large vesicular or hyperchromatic nuclei and a rather abundant dull eosinophilic or basophilic cytoplasm. At times these large cells are in intimate association with a hyaline eosinophilic material of undetermined character. The picture in the simpler proliferative lesions is in agreement with the early lesions of rheumatic endocarditis, and proliferative reactions associated with the hyaline substance bear a striking resemblance to the lesions, as seen in postmortem examination in typical verrucous rheumatic endocarditis. The proliferated cells in the experimental lesion closely resemble those in the rheumatic valve. It is emphasized that the reaction is not exudative, as in bacterial endocarditis. The proliferative reactions described in the valves occurred for the most part at the contact edges, as seen in acute rheumatic fever. In a number of instances small focal accumulations of cells were seen lying beneath the mural endocardium, within the muscle, or at the zone of attachment of the mitral valve. These reactions, essentially large cell proliferative lesions, recall the Aschoff reaction of acute rheumatic fever. These lesions differ from the accumulations of lymphocytes or of lymphocytes with a few large mononuclear cells and foci of more or less mature fibroblasts occasionally encountered in control animals or in animals with simple infection. Well defined proliferative reactions of large and, at times, multinucleated cells in the epicardial and pericardial tissues were observed in several instances, occurring both in uncomplicated scurvy and in scurvy with superimposed infection.

Discussion

(Dr. E. T. Bell, Minneapolis.) The valvular lesions demonstrated, I think, are indistinguishable from those of acute rheumatic fever.

(Dr. B. J. Clawson, Minneapolis.) I should like to compliment Dr. Rinehart on his experimental work. Those lesions which he showed on the valves correspond in every way to the lesions found in acute rheumatic fever. The hyalinized material is like that in the valve in acute rheumatic fever. I think this is the best piece of work on experimental lesions in heart valves that I have seen described.

CELLULAR REACTIONS TO MALARIA (*Plasmodium brasilianum*) IN PANAMANIAN MONKEYS. William H. Taliaferro (by invitation) and Paul R. Cannon, Chicago, Ill.

Abstract. The death and removal of malarial parasites during the course of initial infections and during immunity to superinfection can be directly correlated with the activity of the macrophages, chiefly of the spleen and liver, and to a much lesser degree of the bone marrow. The macrophages of the other tissues are involved only to a very small degree, if at all.

During the acute rise of an initial attack there is a constant, but comparatively low, death rate of parasites, which is correlated with a sluggish phagocytosis by the macrophages. The initial attack is terminated by a sudden disappearance of the forms from the blood (crisis) and is followed by a low grade infection and eventually by a latent infection in which no parasites can be found in the blood except during temporary relapses.

The disappearance of the parasites at the time of the crisis is correlated with a very striking accumulation of the parasites in the spleen and liver. In sections of the spleen the parasites can be seen to accumulate in the splenic cords, but not to pass into the venous sinuses. This concentration proceeds for about 2 days, when the accumulated parasites are suddenly ingested by the macrophages. Both the concentration and later phagocytosis mark the beginning of the acquired immunity and are observable through the remainder of the infection whenever there are enough parasites to make observations.

When immunity to superinfection is tested by injecting parasites into an animal with a latent infection, the macrophages behave as they do at the time of the crisis. Concentration in the spleen and liver begins immediately and phagocytosis takes place within less than 24 hours.

Concomitantly with the development of macrophage activity there is an increasing hyperplasia of the lymphoid elements, particularly of the spleen, the bone marrow, and sometimes the liver (mantling). The degree of lymphoid activation depends upon the severity and length of the infection. In some heavy infections it was pronounced within 48 hours, whereas in others it was only moderate after 14 days.

The hyperplasia of the lymphoid elements and the increased physiological activity of the macrophages present a striking picture of mesenchymal activation, and are in almost exact agreement with our results on the cellular basis of acquired immunity to avian malaria.

Discussion

(Dr. Paul R. Cannon, Chicago.) This work furnishes an exceptional opportunity to study the effects of an infectious agent in the tissues because of our ability to see the parasites and to determine their numbers and fate in the various organs. I should like to emphasize also that there is a striking similarity between the reactions observed and those described with bacteria. Bacteria injected into normal and immune rabbits are removed much more rapidly from the blood stream and are concentrated much more effectively in the spleen and liver of the immune animals. It seems to me that by studying the distribution and fate of malarial parasites we may get ideas not only in regard to parasitology, but also for the study of such bacterial infections as tuberculosis and syphilis, where there is also unequal localization in different organs, as affected by the immune reaction.

(Dr. E. T. Bell, Minneapolis.) I should like to ask if you were able to stain the protoplasm and chromosomes of these parasites in the tissues, and I wish you would tell us how you did it.

(Dr. James Ewing, New York City.) I was very much interested in this report on a disease which of recent years seems to have attracted less and less attention in proportion to the success of preventive medicine. In the Spanish War in 1898 I had the opportunity to observe large numbers of cases that had the aestivo-autumnal infection, and since most of these were fatal, autopsy material was abundant. In that study I was much impressed with the importance of the bone marrow as a factor in malarial infection, and was somewhat surprised to hear that the authors felt that the bone marrow is less important than the spleen and lymph nodes and liver. However, in one of your sections, the only one you showed of bone marrow, the cellular activity is very great. I found the bone marrow a most important factor in aestivo-autumnal malaria. There was a remarkable activity of phagocytosis and a great massing of parasites, extreme multiple infections in the red cells up to six or seven in one cell, and the persistence of parasites in the bone marrow when they were missing in the other organs. All these observations led me to conclude that the bone marrow has a very important influence on human aestivo-autumnal malaria. The disease in the monkey may make a difference, but I wonder if the authors have paid as much attention to the bone marrow as to the spleen and liver.

(Dr. Ralph Miller, Hanover, N. H.) I should like to ask if there was a lymphocytosis which corresponded to the hyperplasia of the lymphatic tissue of the spleen.

(Dr. Taliaferro, closing.) In regard to Dr. Bell's question concerning the staining we have been using Maximow's combination of hematoxylin and eosin-azur. With this, the chromatin of the parasite does not show up well, but the cytoplasm and pigment are beautifully clear.

In regard to Dr. Ewing's statement we have paid a great deal of attention to the bone marrow and were much surprised that the bone marrow seems so unimportant in this work. The difference between your findings and ours may be associated with the fact that you were probably dealing with terminal infections where if the parasites overwhelm the patient and the liver and spleen defenses break down, the bone marrow comes into play. We were dealing largely with animals that recovered. The very fact that you found parasites surviving in the bone marrow would indicate that phagocytosis was not going on as actively as in the spleen and liver.

In reply to Dr. Miller's question, we did get a striking lymphocytosis in some monkeys, but not in others. This was associated with an actual lymphoid hyperplasia, the extent of which depends upon the length and intensity of the infection. In a short infection only the macrophages come into play. In long infections, on the other hand, lymphoid hyperplasia in all phases may be encountered along with the macrophage activity.

THE NEUTRALIZATION OF POLIOMYELITIS VIRUS BY THE SERUM OF NORMAL ADULTS OF NASHVILLE, TENN. N. Paul Hudson and (by invitation) William Litterer, Chicago, Ill.

Abstract. The serums of 25 normal, adult white residents of Nashville, Tenn., were tested for their ability to neutralize poliomyelitis virus. Twenty-one specimens were virucidal when examined without dilution, and 16 of these neutral-

ized the virus when tested again in a further dilution of 1 in 5. An analysis of the age of the donors in relation to the results showed that those of the upper age group (30 to 44 years) had a higher proportion of neutralizing serums and that more specimens were virucidal in the arbitrarily chosen dilution of 1 in 5, than those of the lower age group (17 to 29 years).

Poliomyelitis has occurred in Nashville and in other parts of Tennessee for many years without, however, the appearance of epidemics. Our experimental results are consistent with the epidemiological features of the disease in Southern states. Before it is concluded, however, that the neutralizing property demonstrated is a specific response to exposure to the virus of poliomyelitis, or whether or not it is a manifestation of serological ripening with maturation of the individual, further observations and experimentation must be made.

The high proportion of individuals examined whose serum neutralized virus gives a basis for experimentation in the use of such serum in poliomyelitis therapy.

Discussion

(Dr. Sidney D. Kramer, Brooklyn.) Dr. Hudson, through his own results, as well as similar results collected from the literature, has shown very well that immunity to poliomyelitis is widespread in the general population. He brings up a point, however, that I should like to comment upon. He suggests, as others have recently, that the immunity as determined by the neutralization test may not be specific in type, but may be a maturation phenomenon. It might be well, until more than the inferential data that have been offered in evidence of such a notion is brought forth, to retain our present concept of immunity for which there is considerable concrete evidence. Sawyer's work on yellow fever and Morales' work on diphtheria indicate clearly that immunity to these two diseases occurs only as a result of exposure to the virus of the disease, and, in the case of yellow fever, no immunity was found in localities where this disease does not exist. In poliomyelitis the evidence is almost as striking. The low incidence of immunity in rural districts, the marked correspondence of immunity to the age distribution of the disease, and the fact that the virus has been found in normal contacts suggesting a carrier factor indicate that the presence of immunity to this disease is a result of previous exposure to the virus.

THE RELATION OF ALLERGY TO RESISTANCE IN STREPTOCOCCIC INFECTION.

B. J. Clawson, Minneapolis, Minn.

Abstract. An experimental study of the relation of allergy to resistance was made. A series of 10 animals was made hypersensitive (allergic) to streptococci. A similar series was made highly immune to streptococci without developing the allergic state.

The rate at which living streptococci were removed from the blood and liver following intravenous injections was compared in these two series and with normal animals. The height of the streptococcic agglutination titer was also compared in the three series of animals.

Fifteen minutes after injection organisms were not removed from the blood stream as rapidly from the allergic animals as from the normal animals. This suggested some harmful factor associated with the phenomenon of allergy. After 2 hours, however, a greater number of streptococci per gram of liver was killed in the allergic animals than in the normal animals. This difference was but slight.

The animals that were made immune without developing allergy, on the other hand, killed organisms in the blood stream and in the liver at a much greater rate than either the normal or the allergic animals.

The findings in the experiments suggest that the allergic state is not necessary in the process of general protective immunity to streptococci. It even seems that allergy may be harmful from the standpoint of its effect upon the phagocytic cells.

Conclusions: 1. Resistance to streptococcic infection can be developed artificially without producing a measurable amount of hypersensitiveness (allergy).

2. The state of streptococcic allergy bears no necessary relation to general resistance.

3. Allergy as related to general resistance to streptococci seems to be a useless and at times harmful concomitant phenomenon.

Discussion

(Dr. Max B. Lurie, Philadelphia.) A study of the correlation between the histological changes and the fate of tubercle bacilli cultured from different organs of the rabbit, normal rabbits as well as rabbits that have undergone a previous infection with tubercle bacilli, showed that the capacity of the tissues to destroy the reinfecting organism was related not so much to the intensity of inflammation as to the degree of acceleration of the inflammatory reaction. The greatest amount of destruction of tubercle bacilli took place in the absence of any but negligible inflammation; that is, if the animal had a very extensive primary lesion and it was reinfectd, that animal destroyed the tubercle bacilli immediately, and in the organs, except for rare minute mononuclear nodules, there was practically no inflammation at the end of 24 hours. An animal with little or no residual primary lesion, which reacted to reinfection with intense inflammation, did not destroy the tubercle bacilli as rapidly as did the animal that reacted with little inflammation.

(Dr. Paul R. Cannon, Chicago.) Has Dr. Clawson cultured the liver and spleen and various organs at as early a period as 5 minutes after infection? In some of our studies we have observed in injecting living organisms intravenously into normal and immune animals that within 5 minutes there are many more organisms per gram of tissue in the spleen and liver of the immune than of the normal animal. In similar experiments an hour afterwards there is just the reverse, as Dr. Clawson has shown. It seems to me this finding emphasizes even more strikingly the ability of the immune tissues to concentrate and also to destroy organisms, because, for example, if an immune spleen contains three times as many organisms at the end of 5 minutes as the normal animal, but at the end of 30 minutes has only one-third as many viable organisms, the killing power of the immune spleen would be nine times that of the normal. I wonder whether or not Dr. Clawson has observed this phenomenon.

(Dr. Reuben L. Kahn, Ann Arbor.) I am very much interested in Dr. Clawson's work because we have been investigating problems of a similar nature during the past 2 years. While I do not question Dr. Clawson's results, I am inclined to question if rabbits should be classified as immune or allergic, depending on whether they were injected intravenously or in the tissues. We believe that the difference in the degree of allergy of the cutaneously and intravenously injected animals is quantitative and not qualitative, and it appears to us that an analysis of the data shown by Dr. Clawson proves this view. We believe, too,

that one can lay down a law that a protein, whether it be bacterial or any other type, when introduced in sufficient quantity into the tissues of an animal, will produce a certain degree of allergy.

(Dr. Clawson, closing.) Dr. Lurie's work which has recently been published seems to agree with my findings on streptococci.

In regard to Dr. Cannon's question — yes, I have tested the number of organisms in the liver, spleen and bone marrow at different times and have found, as you did, that at first a greater number of organisms will be found in the liver in the immune animals than in the allergic animals. With this strain of streptococci all the organisms will disappear from the blood stream in something like 30 minutes. If at that time the liver, spleen and bone marrow are cultured, we shall find a greater number of organisms in the immune animals, but it decreases more rapidly also in the immune animals. I used the 2 hour period because it seemed to me a period that would differentiate more sharply between the two kinds of immune reactions.

In regard to Dr. Kahn's question of immunity and allergy, or the use of the term "immune" and "allergic" animals, I think the criticism is well taken. Of course I mean the animals that were made immune without a measurable amount of allergy, and the animals that were made allergic with a low degree of immunity. Those are the two kinds of animals I was using and my results did show that my allergic animals had some protection. I do not know how to produce allergy without producing some immunity. I do think, however, that a high degree of immunity can be produced by streptococci without producing allergy. The method I used to measure my allergy was the delayed tissue reaction. I injected a measured amount of streptococci subcutaneously. With the animals previously injected intravenously, I got even less allergy, less tissue response than I did in the normal animals. In regard to allergy being harmful, I think most workers in tuberculosis would be very happy if they could eliminate the allergic factor in the pathogenesis of a tuberculous lesion in the lung. Allergy and immunity can be separated. For instance, you cannot transfer allergy passively, while immunity can be transferred passively.

INHERENT FACTORS IN RESISTANCE AND SUSCEPTIBILITY TO INFECTIOUS DISEASES. Leslie T. Webster, New York City.

Abstract. Selective breeding experiments were carried out on several strains of mice to determine the possibility of segregating lines relatively susceptible or resistant to a given infection. Care was taken to ensure that the test animals had had no previous exposure to the specific agents and that they contracted a natural rather than an artificial infection. From the Rockefeller Institute hybrid strain, 37 per cent susceptible to *B. enteritidis* mouse typhoid, lines 85 per cent and lines 15 per cent susceptible respectively were selected. The former were descendants of individuals highly susceptible, the latter of individuals relatively resistant. From white-face and black-and-tan strains, lines 90 per cent and lines 95 per cent susceptible were developed in a similar manner. The selected mice reacted consistently when exposed to spontaneous herd infection. Cross and back-cross breeding of susceptibles with resistants gave results indicating that resistance factors are dominant and not sex-linked. Selected mice, with the exception of the white-face strain, were of average weight and fertility. Resistant mice were heavier but not more fertile than susceptible mice. Tissues of resistant mice, not only at the natural portal of entry but throughout the

body, appeared less sensitive to *B. enteritidis* than those of susceptible mice, suggesting a general rather than local influence of the inherent factors. Finally, the several lines were tested for their relative resistance to nasal instillation of *Pasteurella avicidia*, *B. friedlaenderi*, pneumococci, and louping-ill virus. The susceptible white-face line proved highly susceptible to all, the Rockefeller Institute susceptible lines relatively susceptible to the respiratory bacteria and resistant to the virus, the black-and-tan resistant to the bacteria and susceptible to the virus, and the Rockefeller Institute resistant lines resistant to the bacteria and susceptible to the virus, thus indicating that genetic factors concerned with susceptibility or resistance to infection can operate consistently against a number of, but not necessarily all, harmful agents.

Discussion

(Dr. H. G. Wells, Chicago.) This paper is of great importance from the standpoint of the man doing investigative work in infectious disease, as it brings out strikingly the fact that experiments carried out on animals of unknown pedigree give results that are likely to lead to error. I want to call attention to how this coincides with the work of a Dutch investigator, Hagedorn-Le Brand, who published his results in a journal little known to medical men, and therefore they have not received as much attention as they deserved. In brief, his experience was something like this: a strain of mice was picked up in the United States and another strain in Japan, and they were taken to Java for some work on heredity. After a few crossings had been accomplished, an epidemic infection of staphylococcus origin broke out in the stock, and when it was all over and there were no further deaths among the animals the breeder began to make a charting of his results, which brought out an almost perfect Mendelian result. All of the Japanese stock were dead; not one of the American stock was dead, and of the various hybrids in the stock part were dead and part alive, in almost exactly the Mendelian expectation. This illustrates the fact again that susceptibility behaves essentially as a recessive.

THE DIFFERENTIAL STAINING OF LIVING AND DEAD BACTERIA. Frederick P. Gay and (by invitation) Ada R. Clark, New York City.

Abstract. The action of dye stuffs on bacteria is of importance not simply as a means of their identification but in such fundamental problems as investigation of bacterial structure and physiology, the mode of action of disinfectants and the possibilities of dye therapy, and finally on the protoplasmic changes that underlie life and death processes. The authors have made an extensive study of the Proca-Kayser combination staining method, which in their hands has given extraordinarily great accuracy in the identification of dead bacteria, as contrasted with living bacteria. The only other dye stuff systematically investigated is neutral red, which, properly speaking, is a vital stain rather than a stain that differentiates living from dead organisms and which bears on the matter of destruction of bacteria within phagocytes rather than outside them.

A large list of bacteria has been investigated and for comparison yeast cells and nucleated red blood cells stained by the Proca method and by neutral red. Death of bacteria produced in many different fashions has been found to give uniformly consistent results with the Proca stain, which, subject to natural limitations of technique, has been found invariably indicative of life or death.

Reference will be made to studies of nucleated red blood cells which offer certain analogies to the staining of bacteria. Various forms of hemolysis have been studied here and fruitless attempts made to show demonstrable changes in the staining reaction of specifically sensitized cells. The contrasting effects of death both of bacteria and of nucleated red cells within and without phagocytes will be referred to.

Discussion

(Dr. William H. Taliaferro, Chicago.) I should like to ask if Dr. Gay has tried this on protozoan cysts.

(Dr. Gay.) No, we have not. The only very extensive studies that we have undertaken are studies of the nucleated red blood cells under various conditions that cause hemolysis, and I may say that the same change takes place, for the nuclei of such red blood cells stain blue under normal conditions, and the moment these cells are hemolyzed, the nuclei swell and stain red.

CHANGES THAT MAY BE INDUCED IN THE BEHAVIOR OF *B. Typhosus*, WITH REFERENCE TO THE CRITERIA FOR IDENTIFICATION OF THE SPECIES. Ruth Gilbert and (by invitation) Marion B. Coleman, Albany, N. Y.

Abstract. The properties of strains of *B. typhosus* that have been altered after remaining in media containing immune serum have been correlated with those of cultures that are occasionally isolated from fecal specimens. The cultures from both sources mentioned differ from typical strains of *B. typhosus* in that they produce indol, are not agglutinated in typhoid antisera, are non-motile, and ferment rhamnose. Precipitation tests have demonstrated a serological relation between these two types of cultures, that is, the inagglutinable variants of *B. typhosus* and certain of the strains having similar characteristics which have been isolated from the feces of persons convalescent from typhoid fever, from those suspected of being or known to be carriers of *B. typhosus*, and from patients having enteritis of unknown etiology. These findings suggest that the criteria for the identification of *B. typhosus* may need to be revised.

Discussion

(Dr. Leland W. Parr, Washington.) In the isolation of these organisms directly from the feces, or in the culture media, was it your experience ever to observe mucoid encapsulated forms?

(Miss Coleman.) No, mucoid encapsulated forms of the variant have not as yet been found. Encapsulated strains of *B. typhosus* are occasionally isolated, however.

STENOSIS OF PULMONARY CONUS AT LOWER BULBAR ORIFICE, WITH INTERVENTRICULAR SEPTUM ENTIRE (WITH TWO ILLUSTRATIVE CASES). Maude E. Abbott and (by invitation) W. W. Eakin, Montreal, Canada.

Abstract. The rarity of this combination is pointed out and this and other points of interest discussed. In Case 1, a cyanotic boy aged 12 years, the fetal passages were all closed and the myocardium of the greatly hypertrophied right ventricle was the site of an extraordinary degree of fibrosis and localized obliterative sclerosis of the coronaries. In Case 2 the foramen ovale was widely patent and the constriction was the site of extensive vegetations and of a tag 5 cm. long,

which projected free from the pulmonary artery (*Streptococcus viridans* infection).

Only 2 cases have been found in the literature showing this type of stenosis with *closed ventricular septum*, those of Lafitte and Jackson Clarke.

PHOSPHORUS AND ALCOHOLIC CIRRHOSIS. Frank B. Mallory, Boston, Mass.

Abstract. Alcoholic cirrhosis is characterized by the presence of an acidophilic hyaline network or reticulum in the cytoplasm of liver cells. Many or few cells are affected at a given time, depending on the activity of the degenerative process. The reticulum is colored blue by the Macallum and Fiske-Subbarow methods for phosphates.

Chronic poisoning with phosphorus readily produces in rabbits and guinea pigs a cirrhosis that is characterized by a similar acidophilic reticulum in the liver cells. The dose required ranges from a third to a half of a milligram of phosphorus to the kilogram of animal weight. It is given daily by mouth, dissolved in oil of sweet almonds. A minimum of $4\frac{1}{2}$ months is required. The reticulum stains microchemically like that in alcoholic cirrhosis.

Phosphorus given as a remedy for rickets and to promote callus formation may explain the rare instances of alcoholic cirrhosis in children. For its frequent occurrence in adults and for its evident close relation to excessive indulgence in alcoholic beverages, some other source is demanded. But one suggests itself.

Phosphorus is often present in iron ores and thus gets into pig and cast iron and into the iron and steel vessels and containers made from them. It exists as a phosphide and as an alloy of iron. Disintegration of iron by acids in liquors and alcohol may set free phosphorus from the alloy. It would be preserved in water and especially in alcohol just as it is in oils.

Phosphorus is driven off from solutions containing it by a temperature of 30 to 40° C. It unites with the silver of photographic printing paper and if in sufficient quantity, colors it brown to black. Hydrogen sulphide acts in the same way. The sulphide and phosphide can be differentiated in the following way. Decolorize the paper in a solution of potassium permanganate acidulated with sulphuric acid. After washing in water place in the Fiske-Subbarow solution for 5 to 10 minutes. If phosphorus was present in the fluid, it forms silver phosphide in the paper and then, changed to a phosphate, turns blue; otherwise the paper remains white. Treat with hypo to remove unchanged silver salts, then wash thoroughly in water and dry.

By this method phosphorus has been demonstrated in cast iron and in the tin plate of a tomato can after they had been dissolved in dilute hydrochloric acid.

PHLEBOSCLEROSIS OF THE HEPATIC VEINS, AS ASSOCIATED WITH CHRONIC PASSIVE CONGESTION OF THE LIVER AND CARDIAC CIRRHOSIS. Eli Moschowitz, New York, N. Y.

Abstract. Eight cases of sclerosis of the hepatic veins associated with hypertension of the pulmonary circuit, due to various causes, are reported. The lesion apparently is quite common. In all cases the lesions of hypertension of the pulmonary circuit previously described were present, namely, arteriosclerosis of the pulmonary vessels, dilatation and fibrosis of the pulmonary capillaries. Sclerosis of the hepatic veins is only present when a condition causing increased pressure in the hepatic veins can be predicated, the result in every instance of

hypertension of the pulmonary circuit with right heart failure. It is always associated with passive venous congestion of the liver with and without cardiac cirrhosis. The lesion consists in dilatation of the hepatic veins with thickening of both intima and media and proliferation of the elastic fibers, and is interpreted as compensatory to the increased intravenous pressure. Cardiac cirrhosis is an accompaniment of sclerosis of the hepatic veins, and is interpreted as a capillary sclerosis consequent to the increased pressure in the central veins. A narrow zone of cirrhosis around the vena cava and larger hepatic veins, apparently the direct result of pressure, is described as a usual accompaniment of this lesion.

SOME UNUSUAL SOURCES OF HEMORRHAGE INTO THE GASTRO-INTESTINAL TRACT. B. Earl Clarke, Providence, R. I.

Abstract. A report of autopsy findings in four patients dying from hemorrhage into the gastro-intestinal tract is presented. In no case was the source of hemorrhage diagnosed clinically. In one a primary malignant melanoma of the small intestine was found. The second had an enormous abscess of the liver which communicated with the gall-bladder. The hemorrhage occurred into the abscess from which the blood passed into the gall-bladder and through the cystic and common bile ducts into the duodenum. In the third case there had apparently been an old hemorrhagic pancreatitis with pseudocyst formation. The stomach and transverse colon were firmly adherent to the walls of this pseudocyst. Hemorrhage had occurred into the cyst and from here broken through into both the stomach and the transverse colon. The last case presented an old fibrotic pancreatitis and pancreatic calculi. The stomach was adherent to the pancreas. A sharp calculus had penetrated the stomach wall. Hemorrhage occurred into the stomach at this point of penetration.

THE PRODUCTION OF PERSISTENT HYPERTENSION IN DOGS. Harry Goldblatt and (by invitation) James Lynch, Ramon F. Hanzal and Ward W. Summerville, Cleveland, O.

Abstract. In ten dogs, by the carotid loop method of Van Leersum, systolic blood pressure was determined daily for at least 2 months before, and for from 3 to 9 months after, the constriction of both renal arteries by means of a special clamp devised for the purpose. The clamp permitted the degree of constriction of the vessel to be varied. In some of the dogs the constriction was made great from the beginning; in others it was made moderate at first and subsequently increased. Constriction of one renal artery was followed by a moderate or slight rise of blood pressure which tended soon to return toward the normal level. Following the production of bilateral renal ischemia the blood pressure rose to a varying degree in all of the animals. During a variable period following the constriction of the second vessel very high values were common. No attempt was made in this series of animals to remove the accessory circulation through the capsule of the kidney. Probably due to this accessory circulation the pressure tended to decrease in most of the animals, but in all it has remained elevated well above the normal.

In two dogs, in which the clamping of both arteries was made almost complete from the start, the rise of blood pressure that followed was accompanied by the development of uremia, which proved fatal in a few days. In these dogs urea nitrogen, total non-protein nitrogen, creatinin, and methylguanidin in the

blood kept increasing and the urea clearance and output of phenolsulphonephthalein kept decreasing until death. In the remaining eight animals which have survived for many months the only function test that has indicated some renal damage in a few of the animals is the urea clearance. In one dog that has had persistent hypertension for 9 months following the constriction of the renal arteries the urea clearance has been consistently reduced to about 50 per cent of its own mean normal value. In the remaining animals urea clearance showed less reduction or practically no change from the normal.

In one dog the splenic artery and both femoral arteries were greatly constricted some time before the clamps were applied to the renal arteries, but no rise of blood pressure occurred until after the renal arteries had been constricted.

In one dog the right suprarenal body was removed, the left suprarenal body was denervated and its medulla mechanically destroyed, and the left major and minor splanchnic nerves were sectioned. The blood pressure of this animal showed no significant change until after the renal arteries were constricted, when a rise promptly occurred.

Most of the animals are still alive. The examination of the kidneys of 1 of the animals that died accidentally indicates that constriction of the main renal artery can induce significant changes in the vessels of the ischemic kidney. The changes in the vessels of the kidney and of other tissues of the body are being studied.

These experiments indicate that, in dogs at least, ischemia localized to the kidneys is a sufficient condition for the production of hypertension of a type that resembles that which is associated with arteriolar nephrosclerosis in man.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) I should like to know if there is any evidence of anemia.

(Dr. E. T. Bell, Minneapolis.) It is rather surprising that there was no more trouble with collateral circulation by clamping these arteries. When the veins are treated in this way an enormous collateral circulation develops very soon, which restores the circulation of the kidney. What is the effect if these arteries are clamped completely at first? It is a common teaching that removal of both kidneys is not followed by hypertension. Most investigators seem to think that taking out both kidneys does not cause hypertension. One idea of the fundamental mechanism is that there is first an anemia of the kidney, and then a reflex elevation of the blood pressure.

(Dr. F. W. Hartman, Detroit.) We have obtained somewhat similar results to those reported by trying deep X-ray over one or both kidneys. The kidneys in the experiments directed toward the production of hypertension are raised through the abdominal wall, and a lead plate is introduced between the kidney and the body of the dog, and then the kidney is exposed to very heavy doses of X-ray. These experiments have been reported in the literature for some time, and we got a persistent hypertension, such as Dr. Goldblatt has described. The hypertension does not occur, however, when only one kidney is treated. If one kidney is allowed to remain normal we have never yet seen appreciable and persistent hypertension, and I think that many of Dr. Goldblatt's experiments suggest the same thing, that when he only clamped one kidney there was very little, if any, persistent hypertension. That appeared from his charts.

Another angle here is that there is very little correlation between the kidney

function and the hypertension. All of these dogs treated with X-ray died eventually of kidney insufficiency but the hypertension, both diastolic and systolic, occurred before the terminal stage. In fact, the blood pressure nearly always fell in the terminal stages. The animal nearly always has a poor appetite, eats very little, begins to lose weight, and then the hypertension nearly always falls.

As to the methods of taking the blood pressure, we tried the cuff method, as Dr. Goldblatt mentioned, but in most of our dogs we found these cuffs ulcerated through and broke down as the hypertension reached the maximum, and we lost several of our best hypertension dogs from this ulceration and hemorrhage, so that I do not think this method, at least in our work, turned out very well. We used a cuff on the dog's legs similar to the ordinary mercury manometer, also maximum and minimum mercury manometers, and direct blood pressure readings were obtained. Our normals in dogs were a little lower than those recorded by Dr. Goldblatt, from 125 to 130, and I should like to ask him about the training of his animals — how they were handled in getting them in and out of the cages. We decided that blood pressure readings were of very little value unless the dogs had gone through a long period of training and were perfectly coöperative in that they allowed themselves to be lifted out of their cages, taken into the laboratory and laid down on a warm blanket, covered, and then kept perfectly still during the observation of the blood pressure. If there is any break in that technique, or if they shiver, or move on the table, the readings are absolutely worthless, in our experience.

The other thing I should like to ask about is whether there are corresponding changes in the vascular system, particularly in the heart. Was there any hypertrophy of the heart? We found in our group of animals we were able to produce heart-weight body-weight ratios practically double the normal, and the hypertrophy was readily demonstrated with a six-foot cardiac plate. I think that would be worth while to have confirmed in these experiments.

As to the eyegrounds, we also got extensive exudation which was readily demonstrated.

(Dr. Bell.) I think the cuff method gives higher readings on the normal, but it should be of comparative significance when hypertension is produced.

(Dr. Goldblatt, closing.) In answer to Dr. Krumbhaar's question we have not at the present time studied the blood; that is part of the program planned but it has not yet been done.

In answer to Dr. Bell it has been the observation of every physiologist that in acute experiments clamping of both renal arteries causes no significant change in the blood pressure. However, continued determination of the blood pressure of such animals for several days, until death, has not hitherto been carried out. In our experiments when the constriction of both renal arteries was very great, amounting to complete clamping, in so far as the blood flow in the kidneys is concerned, the blood pressure did rise and remained at very high levels until death. In such animals uremia accompanied the rise of blood pressure and proved fatal. The functional changes in our dogs that survived for a long time were less severe than in Dr. Hartman's animals.

In answer to Dr. Hartman, — I am very familiar with his experiments and with those of other investigators interested in experimental production of hypertension. None of the experiments hitherto reported used the method we have just described and which limits the possible cause of at least one type of hypertension to renal ischemia alone. On this account I did not take the time to refer to all the experiments that have been performed on the production of hyper-

tension in animals. In his experiments Dr. Hartman produced severe generalized damage to the kidney by means of roentgen rays. He was not dealing with the exclusive effects of renal ischemia. In some of our animals, after the constriction of one renal artery, there was a rise of blood pressure which did not persist unless the other artery was also constricted. This was to be anticipated if renal ischemia is to be considered the exclusive cause of this type of hypertension. This is in keeping with the knowledge we have of so-called vascular hypertension in man.

I am pleased to have the opportunity to say something about the method used for the determination of blood pressure. The animal was removed from its cage by the same assistant and carried into the room where the testing was done. After a period of rest in the box the observations were made. Ten observations of systolic blood pressure were made at one sitting every day. The mean of these 10 observations is recorded as the daily value in the charts which you have just seen. By taking the blood pressure immediately after the dog was put into the box higher values could have been obtained, but we recorded the 10 values only after a fairly uniform level had been reached. The figures are therefore probably low rather than high.

We have never observed ulceration of the carotid loop after the surgical incisions healed completely and observations of blood pressure were started. In one dog which we have observed at intervals for over 5 years, and which has had a normal blood pressure during the greater part of that time, the loop is still quite intact. Recently we clamped the renal arteries of this dog; the blood pressure has risen and the loop continues to be normal.

About the lower values for normal blood pressure in Dr. Hartman's animals I have little to say except that it is probably explained by the difference of the methods used and the site at which the pressures were taken. I do not consider this difference of significance in this connection as long as the same method is used throughout the experimental period. Since the change in the level of blood pressure and not the absolute value is the significant thing in these experiments, the result should be the same no matter by what method the blood pressure is determined.

In the beginning, during the control period, some of the animals were nervous and this perhaps raised the level of the normal value. I indicated in the charts that there was a tendency during the 2 or more months of the control period for the blood pressure to fall moderately. By the time the clamps were applied the animals had become completely accustomed to the observer so that the factor of nervousness played no part in the striking elevation of blood pressure which followed the constriction of the renal arteries.

About the changes elsewhere in the body, that is, the appearance of the vessels in other tissues and a change in the size of the heart, we have as yet little to say. We have been taking roentgenograms of the chest, and in the dog that has had persistent hypertension for 9 months following the clamping of the renal arteries there is some indication of hypertrophy of the heart. I do not believe that one can interpret the roentgenographic silhouette in quantitative terms as far as cardiac hypertrophy is concerned. This will be done best at autopsy. At the present time the dogs are all alive, except those in which both renal arteries were almost completely clamped at an interval of only a few days and which survived for too short a period to permit of any significant cardiac hypertrophy. This phase of the study is being continued in collaboration with my colleague, Dr. Alan R. Moritz.

BENIGN EPITHELIAL INVASION. William Boyd, Winnipeg, Canada.

Abstract. Epithelial invasion of deeper tissues is not necessarily malignant. The most marked examples of extensive epithelial invasion of a benign character are seen in the gall-bladder in cholecystitis glandularis proliferans. A case is described in which there is a possible change from a benign to a malignant condition. Benign epithelial invasion occurring in the stomach, uterus and Fallopian tube is also discussed.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to ask if Dr. Boyd thinks adenomyoma of the uterus is of this type, or does he think that it is a congenital condition?

(Dr. F. W. Hartman, Detroit.) I have a specimen of a typical papillary adenocarcinoma arising in this condition which I will be glad to send Dr. Boyd.

(Dr. George Baehr, New York City.) I should like to ask if Dr. Boyd considers the crypts of Luschka in the gall-bladder an abnormality, or part of the normal histology of the organ.

(Dr. Boyd, closing.) I do consider them as abnormal, that is to say, I do not believe we ever find them in a perfectly normal gall-bladder. All those gall-bladders in which they are found show recent or old inflammation.

In regard to the question concerning adenomyoma, I do not imagine there is any direct relation between the two conditions. I imagine the adenomyoma is an entirely different process.

THE ANGLE OF THE MITOTIC SPINDLES IN MALIGNANT CELLS. Shields Warren, Boston, Mass.

Abstract. Measurements of the angle included at the centrosome by the outer fibrils of the mitotic spindle indicate that these angles may be fairly constant for different types of malignant cells. Measurements were made with the eyepiece goniometer and also through direct measurement of enlarged photomicrographs. It is necessary to be sure that the two apices of the spindle lie in the same plane, as rotation would give a false widening of the angle.

Measurements were made in 15 malignant tumors: 6 carcinomas, 4 fibrosarcomas, 2 osteogenic sarcomas, 1 melanoma, 1 glioblastoma, and 1 Ewing's tumor. The mean values obtained for the angles were: carcinoma 91° , melanoma 56° , fibrosarcoma 72° for narrow angles and 108° for wide angles, osteogenic sarcoma 69° , and Ewing's tumor 88° .

Discussion

(Dr. Stanley P. Reimann, Philadelphia.) I should like to ask Dr. Warren if he has any data on the mitotic angles in normal growing tissue, regenerating tissue, or in developing fertilized ova.

(Dr. Warren, closing.) I am working on this at the present time. I have a scattering of measurements, rather too few to say anything about for granulation tissue, and there, too, there seems to be a fairly consistent narrow angle, around 60° . I have a number of measurements from normal lymph nodes that show a range of 30° to 45° , centering about 43° .

A THEORY OF PIGMENTED MOLES. THEIR RELATION TO THE EVOLUTION OF HAIR FOLLICLES. George F. Laidlaw and (by invitation) Margaret R. Murray, New York, N. Y.

Abstract. Pigmented moles of human skin follow a peculiar pattern. Usually they are elevated above the general surface. There is a local accumulation of pigment. In the corium there are solid cords and clusters of nevus cells which are associated intimately with the nerves of the skin (Masson, Stout, Foot, Laidlaw). The nerves run in cords and clusters of nevus cells; here and there a nerve terminates in delicate branches which are applied to one or more nevus cells after the manner of Merkel-Ranvier tactile cells.

The most striking aspects of this pattern are two in number. First, it does not resemble anything known in normal mammalian skin. Secondly, it is a faithful reproduction of the pigmented, elevated and innervated tactile spots of the amphibia and reptiles. In the course of evolution the pigmented tactile spots of the amphibia and reptiles were replaced in mammals by hair follicles and by mammalian tactile corpuscles. The pigmented, hairy mole appears to be a link or transition from pigmented tactile organs of the amphibian-reptilian type to hairy tactile organs of the mammalian type. The pigmented, hairy mole is part mammalian and part reptilian. In its hair follicles it is mammalian; in its pigmentation, elevation and the groups of innervated nevus cells in the corium it follows the amphibian-reptilian pattern.

This evolutionary hypothesis offers an explanation of several hitherto obscure features of pigmented moles. It explains the peculiar and constant association of a pigmented elevation of the skin with hair follicles. It explains Unna's *Abtropfung* as a recapitulation of the formation of a reptilian tactile spot, in which tactile epithelia from the basal layer of the epidermis sink down into the corium, separate from the epidermis, and form connections with nerve fibers. It confirms the probability of Masson's view that nevus cells are a mingling of cells from the epidermis with Schwann cells from the nerve fibers. It explains the curious profusion of tactile corpuscles in pigmented moles of the scalp where normally no tactile corpuscles are found; for hair follicles and mammalian tactile corpuscles are parallel evolutionary products of the reptilian tactile spots (L. Plate). Finally it explains why pigmented moles are rare in hairy mammals. Their skin has specialized far beyond human skin in the production of hair. Tested by mammalian standards, human skin is a fetal skin (Bolk, Keith). In its relative absence of hair it is closer to the ancestral reptilian skin and consequently, as in its pigmented moles, it bears more vestiges of former amphibian-reptilian life.

Vascular nevi may be explained as vestiges of the rich capillary plexus of an amphibian skin, introduced at the stage when man's ancestors, the most primitive amphibia, breathed through the skin. Such a capillary plexus may be seen to-day in the skin of the lungless salamanders, the modern representatives of these first land vertebrates.

Discussion

(Dr. Nathan Chandler Foot, New York City.) I think we have to congratulate Dr. Laidlaw on the completeness of everything that he has done in this connection. The chief objection to our work with silver impregnation at the present time has been that it has been incomplete. We have seen a nerve here, and an end organ there, but we have seldom been able to link up the entire picture.

Dr. Laidlaw's remarkable method has done this, so that I do not think anybody can take exception to his pictures. Last year I sent some specimens of silver impregnation to Professor Miescher, who has been the chief contemporary exponent of the epidermoid origin of melanoma; his impression was immediately that the impregnation was incomplete, and his criticism was just. I think if Dr. Laidlaw would send him some of his impregnations there would be very little come-back. Dr. Laidlaw's work is not only complete in respect to the method, but in respect to the fact that he has brought in comparative anatomy to solve the problem, and I can bear testimony to the fact that we often find associated in the same nevus vascular and pigmented nevi, which further bears out the reptilian analogy.

(Dr. E. T. Bell, Minneapolis.) Part of the difficulty of the ordinary pathologist is that he is not able to stain things so beautifully as Dr. Laidlaw does. When we try to stain neurites and axones we get collagen and reticulin and a lot of other things. I should like to ask Dr. Laidlaw to tell us how he uses the silver stain, if this can be done simply.

(Dr. Laidlaw, closing.) The technique is very simple. These sections were stained with our 2 minute technique, based on the Gros-Bielschowsky. Tissue is fixed in neutral formalin. Frozen sections are put in 40 per cent silver nitrate for 2 minutes, then without rinsing placed in 5 per cent formalin for 2 minutes, then without rinsing placed in 5 per cent ammoniacal silver for 2 minutes, and it is done.

THE MOBILIZATION AND EXCRETION OF MELANIN IN NORMAL AND PATHOLOGICAL CONDITIONS. Victor C. Jacobsen and (by invitation) G. H. Klinck, Jr., Albany, N. Y.

Abstract. The normal distribution of melanin in the human is confined to the skin, the pigment layer of the retina, the ciliary body and the choroid, except for its presence in certain parts of the central nervous system (Bloch). Increase in the amount of melanin in these locations occurs in various pathological conditions, being especially pronounced in the malignant melanomas. The isolation of melanin in a pure state is apparently impossible at present because of its relative insolubility *in vitro* without the aid of strong acid or alkali. It must be soluble in certain of the body fluids because it or its precursor, melanogen, colors the urine in many cases of melanoma, and while it is phagocytized by various endothelial cells it has rarely, if ever, been detected in particulate form in the cells of the circulating blood. Since melanin is a constant component of many cells of the body, it follows that the pigment must be disposed of in some way when melanophores die. Also, if this be true, there is much more melanin to be disposed of in conditions of increased melanosis. If the excretion of melanin is interfered with it should be found retained in the body, especially in organs likely to have excretory powers.

In order to determine the manner of disposition of melanin normally and in diseased conditions we have studied by special methods tissues from the following sources: old persons, both blondes and brunettes, adult negroes, individuals with Addison's disease, those with benign and malignant melanoma, and about 400 white mice, each bearing a melanoma derived from one originally discovered by Harding and Passey of Guy's Hospital in 1925, and obtained by us from Sugiura in 1931.

In cases of pathological increase of melanin in the human, such as in Addison's

disease or melanomatosis, the kidneys in all instances contained much melanin in the epithelium of Henle's loops and collecting tubules, and frequently as well in the reticulo-endothelial cells of the liver, spleen, and lymph nodes. Melanin granules were often present in renal casts and one diagnosis of Addison's disease was made partly on the basis of such a finding. In the adult constipated negro melanin was regularly found in small amounts in these same sites in the kidney. Also the kidneys of old people who died of severe wasting disease showed similar findings. White mice harboring melanomas also revealed this distribution of the pigment. In melanosis coli the pigment in phagocytes in the colonic mucosa seems to be true melanin and occurs only in very constipated people.

The melanophores, or cells that can only carry pigment and not produce it, are of several types, among them being (1) macrophages, (2) endothelial cells, and (3) fibroblasts. The main paths of excretion are the kidneys and the intestinal tract, although the skin itself by desquamation doubtless releases much melanin from its own cells. The argyrophilia of melanin after thorough extraction with lipid solvents suggests the best method at present for demonstrating melanin, and Foot's silver bromuration method has been used considerably in this study. The dopa reaction is invaluable in differentiating melanophores from melanoblasts.

The pigment in the zona reticularis of the adrenal appears to be melanin and it is suggested that the pigment is adsorbed by the epithelium after its release by the sympathetic cells of the contiguous adrenal medulla. The pigment of heart muscle is not melanin, according to our present criteria. These studies show that whenever a pigment can reasonably be assumed to be melanin its origin from cells of neuroectodermal origin is quite definitely indicated. Hence, we feel in line with Masson's dictum that melanotic tumors are of neurogenic derivation; the time has come to state unequivocally that melanin itself, no matter where it is found, is always of neurogenic origin.

RENAL LESIONS ASSOCIATED WITH MULTIPLE MYELOMAS. E. T. Bell, Minneapolis, Minn.

Abstract. Proteinuria occurs in the great majority of instances of multiple myeloma, and renal insufficiency is frequently demonstrable in the advanced stages of this disease.

Seventy-nine reports from the literature are reviewed in which the author gave information concerning blood pressure, renal function, or structural alterations in the kidneys, and 11 new cases are reported in which the histological changes in the kidneys are described.

In 4 cases no protein was found in the urine, and in 8 cases serum protein was demonstrated in the absence of Bence-Jones protein. In 60 of 74 cases, 81 per cent, in which a test was made, Bence-Jones protein was found in the urine.

In some instances structural changes in the kidneys are caused by arteriosclerosis, in others, by pyelonephritis resulting from compression of the spinal cord or prostatic hypertrophy. Renal insufficiency may be caused by lesions of these types.

The only direct effect of multiple myeloma on the kidneys is the formation of casts of Bence-Jones protein which obstruct the tubules and cause tubular atrophy. When a large number of tubules are obstructed extensive atrophy of the cortex and renal insufficiency ensue. There is no evidence that Bence-Jones protein injures either tubules or glomeruli. Cortical atrophy is due to tubular

obstruction by casts and should not be interpreted as a nephrotic contracted kidney.

In one instance renal insufficiency was apparently caused by the accumulation of a highly concentrated protein solution in the glomerular capillaries.

Discussion

(Dr. Victor C. Jacobsen, Albany.) In 1917 I reported what was regarded as the first case of multiple myeloma with Bence-Jones protein in the blood serum. It was a case of a Chinese, 60 years of age, who had typical multiple myelomas, with Bence-Jones protein in the urine. In inactivating his serum for the Wassermann test a tremendous precipitation occurred, and further analysis showed he had 7.6 per cent of the Bence-Jones protein in his blood serum. At autopsy the tumors were plasma cell myelomas. He also had very small, arteriosclerotic, granular kidneys. The Bence-Jones protein was largely dammed back in the blood stream. Since that time Bence-Jones protein has been found in the blood serum in small amounts in hypertension and in other non-neoplastic conditions, and we are beginning to know more about it. I should like to make one plea, and that is for the use of the term Bence-Jones protein. It is a protein and hence should not be spoken of as Bence-Jones "bodies," and editors should coöperate in eliminating the latter term from future literature.

(Dr. F. W. Hartman, Detroit.) Regarding Bence-Jones proteinuria, I think in the Tumor Registry of the American College of Surgeons only about 50 per cent of the cases show this reaction. It may or may not be due to the poor technique, but we have been surprised in our own cases how frequently the reaction is repeatedly negative on histologically proved cases, and also how occasionally it is demonstrated in other types of bone tumors. The microscopic sections here, particularly of the glomeruli and the glomerular capillaries, interested me because we have seen similar pictures in a group of dog kidneys where the fundus of the urinary bladder has been anastomosed to the intestine, and we get the urine excreted, and re-absorbed, and re-excreted, holding the non-protein nitrogen level at about 100 to 120 mg. We get in the urine very soon a constant albuminuria, and histological examination of these kidneys shows a very marked dilatation of the tuft capillaries; a little later they fill up with this granular protein and appear exactly the same as the pictures that Dr. Bell has shown; still later this granular material escapes — in some cases you can see the wall of the capillary ruptured, and the granular material in the capsular space. Associated with the glomerular changes we see in these animals typical nephrosis in the first 5 or 6 months and still later they go on to extensive fibrosis. I should like to ask Dr. Bell if he thinks these kidneys are typically nephrotic in the early stages, if he has had an opportunity to examine them in the stages where the glomerular changes are seen.

(Dr. Wiley D. Forbus, Durham, N. C.) We have had occasion to study just such a case as Dr. Bell has described, along with two or three other cases in which Bence-Jones protein was excreted in considerable quantity in the urine. Furthermore, we have been engaged for some time in an experimental study of the excretion of this substance through normal kidneys, and I think I can say at this time, as I have said already in a preliminary report which was published in the *Southern Medical Journal*, that our findings are identical with those of Dr. Bell, in so far as the morphological changes in the kidney tubules are concerned. In the case in which we found the most extraordinary obstructive

tubular changes we were unable to find any functional renal disturbances. We were also unable to find any lesion such as that described by Dr. Bell in the glomerulus. In view of his observation in this connection we shall go back and make another attempt to demonstrate some disturbance there. Experimentally we have done this. We have taken purified Bence-Jones' protein and introduced it into the animal's peritoneum. It is rapidly absorbed when so introduced and that absorption is followed quickly by the excretion of the substance in the urine. It is excreted by the kidneys in an unchanged form. Quantitatively about as much comes out as goes in, although it is said by some that a certain amount of the substance is destroyed. We have been unable to produce in dogs and in rabbits the obstructive tubular changes in the kidney that we have observed in the human being. However, we have succeeded in producing very definite changes in the epithelium of the proximal parts of the tubules, which we think probably are definitely related to the Bence-Jones protein. This work is still in the process of development and that is all we can say at the present time. We hope in a short time to have more data.

(Dr. Bell, closing.) There is no doubt that there are occasional cases in which uremia is due to arteriosclerosis, or to pyelonephritis. However, these are in old men who have an hypertrophied prostate and arterial disease, and I am inclined to think these are associated lesions and not due to the myeloma.

As to the incidence of Bence-Jones protein in the urine Magnus-Levy gave it as about 70 per cent and Geschickter and Copeland about 60 per cent of the cases. I think that is somewhere near the proportion that show Bence-Jones protein. The only case of renal insufficiency referable to myeloma is the blockage of the tubules by casts, aside from the exceptional case of blocking of the glomerular capillaries by protein.

SPONDYLITIS OF SWINE ASSOCIATED WITH BACTERIA OF THE BRUCELLA GROUP.

William H. Feldman and (by invitation) Carl Olson, Jr., Rochester, Minn.

Abstract. Lesions of a specific spondylitis were obtained from 24 different swine and organisms of the genus *Brucella* were secured by culture procedures and by animal inoculations from 10. The affected swine were apparently symptomless and without lesions in other parts of the body. The lesions were encapsulated, abscess-like structures occupying irregular cavitations in the body of the vertebrae. Relation of the lesions of the vertebra to the usual pathology of infectious abortion of swine was not observed.

The presence in these necrotic foci of the vertebrae of *Brucellae* of proved pathogenicity for guinea pigs and rabbits provides a possible source of infection for farmers, packing house employees, retail butchers, and others who may handle pork in an uncooked state.

DEGENERATIVE CHANGES IN JOINTS RESULTING FROM CONTINUED TRAUMA AND INCREASING AGE, AND THEIR RELATION TO HYPERTROPHIC ARTHRITIS.

Granville A. Bennett and (by invitation) Walter Bauer, Boston, Mass.

Abstract. Studies that have been in progress during the past 3 years have demonstrated quite clearly that the ability of articular cartilage to regenerate or repair in surgically created defects, or in defects due to repeated trauma, is at best very feeble and limited. These same studies have further demonstrated

that such intra-articular defects are not in themselves a cause of important joint pathology, such as the hypertrophic changes described by some workers. However, repeated trauma due to displaced patellae did result in certain animals in very marked changes similar to those observed in human hypertrophic arthritis.

Because of these findings we undertook to observe what changes might take place in human joints subjected to unusual use or repeated trauma, as well as to determine what changes resulted from wear and tear of increasing age. For the first part of this problem we studied a single joint, which had been subjected to repeated trauma or constant use, using the opposite joint as a control. In such instances one finds constant use or repeated trauma is of itself adequate cause for marked hypertrophic or degenerative changes. For the second part of the problem we collected a series of human knee joints representing various ages from the first to the ninth decades. These joints were obtained at autopsy from individuals who, so far as we could determine, had never had symptoms of joint disease. The joints were studied roentgenologically, macroscopically, and microscopically. Such studies revealed that with each succeeding decade in life beyond the second, the knee joint shows increasing pathological changes, degenerative in nature and confined for the most part to the articular surfaces. The earliest, as well as the most advanced changes observed in these joints, were identical in kind to those commonly spoken of as the characteristic lesions of degenerative or hypertrophic arthritis. From this study one is led to believe:

1. That hypertrophic or degenerative arthritis results from the wear and tear of increasing age and repeated trauma and is not the result of an inflammatory process, as some workers have contended.
2. That arteriosclerosis is not an important etiological agent in producing such lesions because either minimal or marked changes may be found in patients with marked peripheral arteriosclerosis.
3. That humans may have marked degenerative changes indistinguishable from hypertrophic arthritis without having important symptoms of joint disease.
4. That if hypertrophic or degenerative arthritis is nothing more than degenerative joint changes due to the wear and tear of increasing age, one might challenge the correctness of the use of the word arthritis in individuals with such joint changes.

Discussion

(Dr. E. T. Bell, Minneapolis.) This is a very good contribution to a subject that is of great interest at present. The lesions which Dr. Bennett showed us are quite like those of so-called arthritis deformans. Do you make a distinction between atrophic and hypertrophic arthritis, or do you think these are simply variations of the same disease?

(Dr. Bennett, closing.) We do make a distinction between Type I, atrophic, and Type II, hypertrophic arthritis. As this study would indicate, one is probably correct in believing that the joint lesions in hypertrophic arthritis are degenerative in nature and are not the result of infectious or toxic agents.

THE JOINTS IN EXPERIMENTAL SCURVY AND IN SCURVY WITH SUPERIMPOSED INFECTION. WITH A CONSIDERATION OF THE POSSIBLE RELATION OF SCURVY TO RHEUMATIC FEVER. James F. Rinehart and (by invitation) S. R. Mettler, San Francisco, Calif.

Abstract. The studies on the joints in scurvy, and in scurvy with superimposed infection here outlined represent a preliminary report on data that are as yet

incomplete. Animals used in the heart study comprise the material of this report. Joint pains, at times swelling and hemorrhage, are well known to be a part of the symptomatology of scurvy. Joint manifestations were frequently observed in the scurvy animals, both infected and non-infected, in the various series of experiments performed. It is considered significant that of the entire group of 18 animals subjected to streptococcus infection, and given the basic diet with added orange juice, in not a single instance was joint involvement observed.

Particular attention was paid to the joints in the last experiment conducted. In this experiment there were 4 animals maintained on a diet deficient only in vitamin C, in a condition of chronic scurvy without infection, 7 animals with streptococcus infection maintained on an adequate dietary, and 9 animals on the same chronic scurvy dietary as the first group, but which had been infected 22 days after the onset of the experiment. Observation on the joints was made in the entire group 31 days after the onset. Of the 4 animals in the scurvy group 2 showed some stiffness and partial fixation of the knee joints. Of the 7 animals infected, but receiving an adequate vitamin C supplement to the diet, no involvement of the joints was observable. Two animals showed partial fixation of the knee on the left side due to the local cutaneous and lymph gland infection, but no joint involvement. Of the 9 animals subjected to both the chronic scurvy dietary and infection, 8 showed partial fixation of one or both knees and 3 in addition presented marked swelling of one or both knees. Five days later another animal showed mild but definite swelling of both knees. In one instance the swelling was due to a frankly suppurative arthritis, in the others not.

It is believed that these observations, although consisting of a relatively small number of animals, show a definite tendency for arthropathy in the scurvy animals and a definitely increased tendency for arthropathies in the scurvy animals subjected to a superimposed infection. In the guinea pigs on adequate dietary the infecting organism used (*Streptococcus hemolyticus* of spontaneous lymphadenitis) showed no tendency whatever to localize in or produce lesions in joints.

One or more joints were examined histologically in 30 animals as follows: acute scurvy 3, subacute or chronic scurvy 4, acute scurvy plus infection 1, chronic scurvy plus infection 8, infection followed by scurvy 4, control dietary without infection 3, and infection with adequate diet 7. The control animals and those with infection and maintained on an adequate dietary showed no pathology in the joints, synovia, or joint capsules. The animals subjected to acute scurvy presented a little fibrinous hyaline material in the joint cavity, slight or no synovial proliferation, some hemorrhage, or no change in the capsule, and a mild atrophy of the muscle. One animal showed a scattering of polymorphonuclear leukocytes in the joint space. In the group of chronic scurvy animals some hyaline material was found in the joint spaces, and a variable but usually definite proliferation of the synovia. One animal showed a little free blood in the joint space. The capsular tissues presented, as a rule, evidence of hemorrhage and some fibrous and vascular proliferation. The muscles showed edema, degeneration, reactive proliferation, and usually some hemorrhage. The animals subjected to combined scurvy and infection presented changes of a similar sort, but in general more striking with a greater amount of hyaline material in the joint space, usually more intense synovial proliferation, and greater capsular proliferation. The joint symptomatology and pathology described suggest that a condition of partial scurvy may be an important factor in the

genesis of the acute joint manifestations of rheumatic fever or the more chronic disability of the closely allied condition of rheumatoid or chronic infectious arthritis.

Although not looked for in the routine examination of animals a subcutaneous nodule, answering closely the description of the subcutaneous nodule of rheumatic fever, was found in one animal, overlying the fibula. This animal in addition showed a proliferative lesion on the mitral valve and a well defined, localized, pericardial proliferative reaction. In addition the same animal presented partial fixation and swelling of both knees. The knee joint sectioned showed a distinct synovial and periarticular proliferation.

A Consideration of the Possible Relation of Scurvy to Rheumatic Fever: The experimental data cited in which degenerative and proliferative reactions resembling those of rheumatic fever are produced in the heart valves, myocardium and pericardium by subjecting the scorbutic guinea pig to infection with streptococci, together with the data indicating an increased tendency of the scorbutic animal under the influence of infection to arthritic symptoms and pathology, naturally led to a careful analysis of other data which might support the concept that scurvy may bear a relation to rheumatic fever.

The epidemiology of rheumatic fever has long presented a challenge to the medical profession. Certain peculiarities of the disease, notably the social incidence, the age incidence, the seasonal incidence and the geographic distribution await a satisfactory explanation.

Social Incidence: It is a well recognized fact that rheumatic fever is predominantly a disease of the poor. Swift has estimated that it is 15 to 20 times more frequent in the laboring classes than in those forming the bulk of private practice. The greatest incidence occurs among the poor of elementary school age living in the cities. One need only consider the notoriously poor diet of the poorer children of metropolitan areas, such as New York and London, to realize that malnutrition and probably latent scurvy, if it exists at all, would occur under such circumstances. A number of investigators have emphasized the malnourished state of the child that is prone to develop rheumatic fever. Although relatively little attention has been directed to the clinical occurrence of latent scurvy it has recently been demonstrated clearly in Sweden by Falk, Gedda, and Göthlin to be clinically demonstrable in the spring in approximately 20 per cent of the children of elementary school age.

Age Incidence: The age of onset of acute rheumatic fever is in the vast majority of cases under 15 years — the average age of onset has been given at from 7 to 10 years. It is considered significant that it has been shown recently that children of the elementary school age (5 to 14 years) require approximately twice the vitamin C intake per kilogram for prevention of latent scurvy than adults require.

Seasonal Incidence: Suggestive evidence of a possible relation of latent scurvy and rheumatic fever is present in the concurrent seasonal incidence of the two conditions. Rheumatic fever is known to reach its peak of incidence in the spring. This is the period, of course, following a winter of diminished availability of fresh fruits and vegetables, and consequently the period of greatest incidence of scurvy and latent scurvy. Falk, Gedda and Göthlin have recently shown in Sweden that April is the period in which latent scurvy may be detected. In this connection it is of interest to note that a number of investigators have demonstrated that the milk available in winter and early spring (due to dry feed) has one half the anti-scorbutic potency of summer milk. Con-

sidering the importance of milk in the dietary of children this finding would appear of considerable significance.

Geographical Distribution: Some students of the subject have gone so far as to claim a complete absence of rheumatic fever in the tropics. All agree in its extreme rarity in this zone. Scurvy is rare in the tropics. An analysis of the dietary in the tropics would appear to give adequate reason for this. Although varying in different parts the foods eaten would appear to give an abundant supply of vitamin C.

Symptomatological Similarities: A very suggestive parallel exists in the symptomatology of the conditions of latent scurvy and of the prerheumatic or early rheumatic state. We find general under-nutrition, fatigue, loss of appetite, loss of weight, myopathy, or pains in the limbs and chest, growing pains, irritability or nervousness, and anemia as symptoms in common. The most accurate index for recognition of latent scurvy appears to be an estimation of the capillary resistance. A diminished resistance is characteristic of latent scurvy and has been recorded in acute polyarthritis.

It is suggested that latent scurvy prepares the soil or provides the susceptible host in whom with the added insult of acute infection develop the characteristic train of symptoms and pathological evolution of acute rheumatic fever. An adequate explanation would appear to be present for the variety of organisms, notably various forms of streptococci, that have been implicated as the etiological agent.

The rather highly suggestive experimental data presented, together with the data offering an explanation for the peculiar epidemiology of rheumatic fever, would indicate a possible if not probable relation between latent scurvy and rheumatic fever. It would appear that further experimental and clinical research should be conducted to verify or refute this relation.

Discussion

(Dr. E. T. Bell, Minneapolis.) Dr. Rinehart is offering evidence that defective diets are a factor in the origin of rheumatic fever. We may of course accept this and still believe that rheumatic fever is most frequent in those exposed to cold and dampness.

SUSCEPTIBILITY TO PNEUMOCOCCUS INFECTION AS MEASURED BY SPECIES SPECIFIC AGGLUTININS. Theodore J. Curphey, New York City.

Abstract. A survey has been made of over 200 normal rabbits to determine the presence of species specific agglutinins to R pneumococci. For this purpose 5 R strains have been used in the tests. It was found that the animals tested could be divided into three groups: those having no agglutinins, those showing agglutinins in low to moderately high dilutions, and those showing agglutinins in relatively high dilutions. Following this these animals were infected intradermally with a constant dose of a smooth Type I *Pneumococcus* of high and constant virulence, and blood cultures taken at the end of 24 hours. The susceptibility of the animals was measured in terms of the bacteremic level and the extent of the local lesion at this time interval. An analysis was then made for the purpose of determining if any correlation existed between the concentration of R agglutinins and the degree of bacteremia or the extent of the local lesion. Many of the animals were treated with immune serums in comparable doses at this interval after infection, and further studies were made of the variations of

the agglutinins to the original R strains and the infecting S strain, until death or recovery took place. Following recovery the animals were tested at varying intervals for R and S agglutinins. Reinfection experiments were also carried out with observations on the variation of the R and S agglutinins. Attempts were also made to enhance natural species specific immunity by immunization with R pneumococci. These immunized animals were infected with S strains and observations made on the variation of the R and S agglutinins.

Discussion

(Dr. Francis G. Blake, New Haven.) I should like to ask the age and size of the rabbits used, because Dr. Curphey has reported a very much higher incidence of species agglutinins than we have found in rabbits weighing from 1000 to 1500 gm.

(Dr. Curphey.) The animals were chosen from the general run of stock, no attempt being made to control their age. The weights were relatively constant; they varied from 1800 to 2400 gm.

CATAPHORETIC TIME AND VELOCITY OF STREPTOCOCCI AND PNEUMOCOCCI AS ISOLATED IN STUDIES OF THE COMMON COLD, INFLUENZA, BRONCHOPNEUMONIA, AND LOBAR PNEUMONIA. Edward C. Rosenow, Rochester, Minn.

Abstract. The cultures from nasopharynges and sputum of patients suffering from colds, influenza, bronchopneumonia and lobar pneumonia, from the nasopharynx and teeth of well persons, from milk, and from inoculated animals, during and following epidemics of colds and influenza, were made on blood agar plates and in dextrose brain broth. The cataphoretic time and velocity of streptococci from dextrose brain broth cultures were determined in distilled water with the Northrop-Kunitz-Mudd apparatus, and the virulence was determined by injection into animals.

The slowing action on the respective streptococci of the serum of persons during and following attacks of the respective diseases and of well persons was studied cataphoretically.

Distribution curves of cataphoretic time and velocity of the streptococci or pneumococci isolated in these diseases were found fairly characteristic in each disease studied as these occurred in epidemic waves during the last 3 years. The cataphoretic time and velocity of streptococci isolated from the nasopharynx and teeth of well persons, of persons ill with chronic diseases, and from raw milk during epidemics of the common cold and influenza commonly resembled, sometimes markedly, that of streptococci isolated from patients suffering from colds and influenza. As epidemic waves disappeared the cataphoretic time and velocity of streptococci isolated from the nasopharynx of persons after recovery from colds and influenza, and from the nasopharynx and teeth of well persons, and from milk, became like that of streptococci isolated from the nasopharynx of well persons in non-epidemic times, and in persons ill with certain diseases it became characteristic of the disease in question.

The serums obtained from persons during convalescence from colds and influenza were found to possess specific slowing effects on the respective streptococci.

The etiological importance of streptococci in colds and influenza is emphasized.

THE ETIOLOGICAL RELATION OF FILTERABLE VIRUSES TO THE PNEUMONIAS
COMPLICATING INFLUENZA, MEASLES AND WHOOPING COUGH. Howard A.
McCordock, St. Louis, Mo.

Abstract. In a study of vaccine virus pneumonia in rabbits Muckenfuss and I have shown that two distinct types of reactions can be produced by different concentrations of virus. A strong virus produces a hemorrhagic and edematous consolidation with occasional irregular areas of necrosis and hemorrhage which resemble infarcts. This acute lesion has been called hemorrhagic virus pneumonia. A dilute virus induces a proliferative cellular reaction, which has been styled interstitial virus pneumonia, because the walls of the bronchi, the alveoli and the blood vessels are infiltrated with large and small mononuclear cells. Compact masses of fibrin and large mononuclear cells are seen in some of the alveoli adjacent to an involved bronchus.

The experimental hemorrhagic virus pneumonia produced in rabbits by vaccine virus is similar to the hemorrhagic and edematous lobular consolidation with foci of necrosis observed in the lungs of individuals dying a few days after the onset of symptoms of epidemic influenza. The infiltration of the interstitial pulmonary tissue in interstitial virus pneumonia in rabbits resembles the accumulation of cells seen in the interstitial bronchopneumonia that so frequently complicates influenza, measles and whooping cough in man. The complete histological picture of interstitial bronchopneumonia cannot, however, be reproduced by the action of virus alone, because the polymorphonuclear exudate in the bronchi and adjacent alveoli is usually lacking. This element can be added if bacteria are used in conjunction with dilute vaccine virus. We have used various types of bacteria, which, when injected alone, produce an ordinary lobular pneumonia.

Since interstitial bronchopneumonia can be reproduced by vaccine virus and bacteria, and because it is found in known virus diseases such as measles, dog distemper and psittacosis, we regard it as the type reaction for the combination of a virus and bacteria, but in no sense specific for a particular virus or a bacterium. A moderate cellular infiltration of the walls of the bronchi, in addition to a lobular pneumonia, is occasionally seen in bacterial infections. This combination is often found in bronchial diphtheria and has been cited as an instance of interstitial bronchopneumonia caused by a bacterium. The cases of diphtheria that we have studied show lymphocytes and plasma cells in the bronchial wall with leukocytes near the necrotic epithelial surface, but the large mononuclear cells are scarce. While we have emphasized the cellular infiltration of the bronchial wall in interstitial bronchopneumonia, it must be pointed out that this is only one element of the pathological change in this type of pneumonia. The compact fibrin masses and the large mononuclear cell exudate in the alveoli surrounding a thickened bronchus are equally characteristic, as well as the new growth of connective tissue that later appears in the infiltrated bronchial walls. While bacteria may call forth a slight cellular infiltration of the bronchial walls, there is no definite evidence that true interstitial bronchopneumonia can be produced by microorganisms. With vaccine virus and bacteria we have been able to reproduce not only all the histological features of interstitial bronchopneumonia, but also many of the pulmonary complications that follow influenza, measles and whooping cough, such as pleurisy, empyema, lung abscess and the proliferation of the alveolar epithelium that was observed so often during the 1918-19 influenza pandemic.

If a virus is necessary for the production of interstitial bronchopneumonia, as we conclude from our experimental work, one would expect to find such an agent associated with the three human diseases that are complicated by this type of pneumonia. Of these diseases measles alone is at present generally regarded as being due to a virus. Although the etiology of epidemic influenza is unknown many investigators have suggested that it is primarily caused by a virus and we regard the similarity of the pulmonary lesions to those of measles, a known virus disease, and to experimental virus pneumonia in animals, as indirect evidence in support of such a view. Whooping cough, on the other hand, is universally regarded as a purely bacterial disease due to the Bordet-Gengou bacillus. Since interstitial bronchopneumonia is found in a high percentage of fatal cases of whooping cough it was necessary to seek evidence of the presence of a virus in this disease, if our conclusion that a virus is necessary for the production of this type of pneumonia was to stand.

Last year I reported the finding of intranuclear inclusions in the lungs of 12 out of 35 children who had died of pertussis and their absence in all but 2 children of a control series of 90 autopsies. Since then 18 out of 40 cases of pertussis (45 per cent) have shown these inclusions. The presence of cellular inclusions in such a high percentage of our cases of whooping cough is strong evidence of the possible association of a virus with this disease. In this connection it is interesting to recall the history of three other diseases, namely, hog cholera, canine distemper, and psittacosis, which for years were regarded as being due to specific bacteria but now are known to be caused by viruses.

The similarity of virus pneumonia in animals to the pneumonias complicating influenza, measles and whooping cough, suggests that viruses are associated with these three diseases, and the presence of intranuclear inclusions in whooping cough strengthens the evidence for the presence of a virus in this disease.

Discussion

(Dr. Francis G. Blake, New Haven.) The theory is obviously a very interesting one. I should like to ask what the character of the cellular reaction is in the interstitial infiltration: whether it is constantly a mononuclear cell infiltration, as I judge it is from the slides, or whether there is any polymorphonuclear interstitial infiltration; also whether you consider the mononuclear character of the cellular interstitial infiltration to be the important point. I raise that question because grossly quite similar pictures of interstitial infiltration without polymorphonuclear exudate in the alveoli or bronchioles can be found in animals injected with bacteria in which no virus has been introduced.

(Dr. McCordock, closing.) Of course the important cellular element in interstitial bronchopneumonia is the mononuclear cell infiltration, which consists of large mononuclear phagocytes, lymphocytes, and occasionally plasma cells. In animals injected with vaccine virus, when strong virus is used, one often sees an interstitial cellular infiltration consisting almost exclusively of polymorphonuclear leukocytes, as one might expect following the destruction of pulmonary tissue. In general, the large mononuclear cell infiltration is characteristically associated with viruses, as was pointed out by Ledingham in a study in which vaccine virus was introduced into the skin, and we believe that vaccine virus calls forth a mononuclear cell infiltration. In addition to this mononuclear cell infiltration, vaccine virus can produce not only the acute lesions seen in influenza, but also the more chronic ones, and by the addition of bacteria to the

virus one may reproduce many of the pulmonary complications of influenza, whooping cough and measles. That is far more than can be done with bacteria of any type, or with any other irritating substance. Winternitz has shown that irritating gases will produce a lesion not unlike the hemorrhagic virus pneumonia we have described, and others have shown that fluid cultures of *B. influenzae* will produce similar lesions, but with neither of these substances has any one ever been able to reproduce the chronic type of interstitial pneumonia, or the pulmonary complications seen following influenza, whooping cough or measles.

FRIEDLÄNDER BACILLUS PNEUMONIA. Charles T. Olcott, New York, N. Y.

Abstract. The clinical and autopsy findings of 5 acute cases of pneumonia proved by blood culture to be due to Friedländer's pneumobacillus are presented. Four of them showed a lobar, the fifth a lobular distribution. All were in males between 38 and 55 years of age, and large fatty livers were found in 3. In 4 cases the white blood count was less than 7000, in 1 less than 2000. In 3 the pulmonary alveoli contained large mononuclear phagocytic cells in greater numbers than polymorphonuclears.

As all the cases were of short duration (2 days to about a week), it is postulated that a primary mononuclear reaction is characteristic of at least some Friedländer pneumonias.

Discussion

(Dr. Francis G. Blake, New Haven.) Among other things it seems to me that this report illustrates the caution one needs to preserve in attempting to relate mononuclear or polymorphonuclear cell infiltration to virus or bacterial infection.

(Dr. Nathan Chandler Foot, New York City.) I think the importance of these monocytes or mononuclear cells in these exudates in the lung is very often overlooked, on account of the subjectivity of the observer. In Germany they have been explained away largely on the basis that they represent desquamation, and for that reason they have been neglected. These are much more common in pneumonic exudates than is generally believed; most people observe them and pass over them, and I think Dr. Olcott's objectivity is to be commended.

(Dr. E. T. Bell, Minneapolis.) Dr. Foot, how do you classify the alveolar cells in tuberculous pneumonia?

(Dr. Foot.) I think they belong in the same category. There is desquamation, but the number of cells desquamated is rather small, as compared to the number of cells migrating into the alveoli.

(Dr. Howard T. Karsner, Cleveland.) There are three questions I should like to ask. (1) Is a low leukocyte count in the blood a phenomenon frequently noted in the reported cases of Friedländer bacillus pneumonia? (2) Were the patients in the hospital for a sufficient length of time so that repeated blood counts could be made to determine whether the leukocyte counts Dr. Olcott reported were continuous or terminal depressions? (3) Did the bone marrow show cytological changes or bacterial invasion?

(Dr. Olcott, closing.) From a survey of the literature I have found three references which quote a leukopenia (Lord, Belk and Fremel *et al*).

With relation to the time in the hospital, the man with the lowest count was

in the hospital only 9 hours, and only one case was in over 2 days. We unfortunately had no repeated blood counts.

With regard to the bone marrow, I have studied only 1 case and in this there was no characteristic departure from normal.

THE GENESIS OF ONE GROUP OF SECONDARY PNEUMONIAS. Virgil H. Moon, Philadelphia, Pa.

Abstract. Pulmonary congestion and edema, not caused by cardiac deficiency, precede the development of a group of secondary pneumonias. Circulatory failure of the shock type produces marked pulmonary congestion and edema resulting from atony of capillaries and venules. Krogh showed that capillary atony and dilatation result from all types of capillary poisons. Severe toxemia of diverse origin causes such vascular atony and permeability to develop gradually. Severe burns, extensive surgical procedures, toxemias of pregnancy, pancreatitis, intestinal obstruction, mesenteric thrombosis, severe jaundice or infections of high virulence may cause circulatory deficiency of this type.

Secondary pneumonia following burns is typical of this group. The lungs are deep red, heavy, edematous, and consolidated in irregularly distributed patches. Fibrin is deficient and the consolidation is less dense than in primary pneumonias. The results of bacteriological studies are not uniform. Frequently mixed cultures result, including combinations of pneumococci, streptococci, staphylococci and *Micrococcus catarrhalis*. Pneumonia following influenza is of this type and is indistinguishable from that following burns or other toxemias.

Toxemias of diverse origin may produce circulatory deficiency resulting in pulmonary congestion and edema. One group of secondary pneumonias results from infection of such lungs with the various pathogenic organisms present in the respiratory tract.

Discussion

(Dr. E. T. Bell, Minneapolis.) It was pointed out by some one in a paper presented at the last annual meeting that cultures from the lungs of practically all postmortem cases are positive. I should like to ask Dr. Moon if that is his experience, and if he thinks we can form any conclusions from the postmortem cultures of the lung. I should also like to ask if he thinks that areas of atelectasis play any part in these secondary foci in pneumonias of this type.

(Dr. Moon, closing.) It is perfectly true that cultures from lungs at postmortem almost always contain organisms. The same uncertainty exists in cultures from Friedländer's pneumonia and from pneumonia following any of the other known infectious agents. Neither is there any characteristic type of organism that predominates in influenzal pneumonia, and I wish to emphasize the analogy between influenzal pneumonia and the pneumonias under discussion. I believe that they belong in this group. This belief is strengthened by the fact that Underhill and Ringer reported an increased concentration in the blood in those cases that ran a rapidly fulminating course. The hemoglobin in such cases ranged from 110 to 140. They found that patients who recovered from influenza had no increased blood concentration.

I have made no observations on the relation of atelectasis to the type of pneumonia that I have presented.

PNEUMONIA IN NEWBORN INFANTS. Margaret Warwick, Buffalo, N. Y.

Abstract. In a series of 240 autopsies upon newborn infants dying during the first 10 days of life pneumonia was found in 46, or 19 per cent. Three of these were discarded because their etiology was evident and they showed a different type from the others. That left 43, or 17 per cent of cases of pneumonia in newborn infants for study and analysis.

Of these 10 were stillborn, 8 lived less than 12 hours, and 6 less than 2 days, making 24, or 56 per cent, which must have developed their pneumonia while yet *in utero*. Two of these were of but 5 months gestation. Twenty, or 46.5 per cent, had no other pathological lesions and, without pathological examination, would have been passed as atelectasis. Twenty-one, or 50 per cent, showed traumatic lesions from difficult births and 2, or 4.8 per cent, showed congenital anomalies.

Only 11, or 25.6 per cent, were born by spontaneous deliveries with an average labor of 13 hours, while the others had interference of some kind. The infants varied in size from 450 to 4225 gm. with an average of 2871 gm. Their positions *in utero* showed a large percentage of abnormal ones. These various abnormalities suggest that there might have been cause for asphyxia, which would stimulate premature respirations that would draw amniotic fluid, which might or might not be infected with pathogenic organisms, into the alveoli of the lungs.

Amniotic fluid, in variable amounts, is a frequent finding in the lungs of newborn infants. This is recognized by the presence of fat, cornified epithelium from the surface of the body, lanugo, fluid and masses of bile salts from the meconium, which are present in various ratios. This foreign material is usually in the midst of an area showing exudation of polymorphonuclear leukocytes. In this series the various constituents of amniotic fluid were found in 175, or 73 per cent of the total series of 240. And in the 43 cases of pneumonia it was present in 37, or 86 per cent.

Gram stains of the lungs showed bacteria present in 11, or 25.5 per cent. The general opinion is that these pneumonias are caused by the aspiration of amniotic fluid which has become infected by pathogenic organisms from premature rupture of the membranes. In this series only 5, or 12 per cent, had rupture of the membranes long enough for such a thing to take place. In 27, or 63 per cent, the membranes had ruptured less than 1 day before birth. In 11, or 25.8 per cent, the time was not recorded. In the 3 cases where Cesarean section was done the membranes were intact until the child was delivered. Therefore, infections of the amniotic fluid could not have taken place unless the bacteria could have passed through the intact membrane or in tiny, unsuspected tears, which is highly improbable.

Atelectasis is a frequent accompaniment of pneumonia in the newborn and is usually recognized, while the pneumonia is not. In this series there were large areas of atelectasis in 20, or 46.5 per cent, small areas in 12, or 28 per cent, and none in 11, or 25.6 per cent. The exudate and foreign material were usually found in the center of the atelectatic areas.

Former observers have thought that bacteria were the cause of pneumonia in the newborn, but this series points toward aspirated amniotic fluid as the etiological factor. It is also possible that either may cause similar lesions. Chemical or mechanical irritation may cause acute inflammation and exudation in other parts of the body. Pneumonia occurs in cases where there is no demon-

strable chance of bacteria entering into the amniotic sac, and it is usually associated with large amounts of amniotic fluid in the lung alveoli. And, whatever the cause, pneumonia in the stillborn and those dying during the first few days of life is a definite pathological entity and should receive more recognition from both obstetricians and pediatricians. It may cause death directly by the exudation or indirectly by preventing proper aeration of the lungs.

Discussion

(Dr. Sidney Farber, Boston.) I think that we ought to differentiate sharply between the reaction in the lungs following aspiration of amniotic fluid which contains vernix caseosa, cornified cells, and so on (the so-called normal amniotic sac contents), and that following aspiration of amniotic fluid that contains meconium. I think we are dealing with two different things. Some years ago we studied the lungs of rabbits following insufflation of amniotic fluid that contained vernix and cornified cells. That study combined with observations on lungs of newborn infants led us to conclude that the amniotic sac contents alone aroused no important reaction in the lungs, or at best a very slow mononuclear reaction. We found no evidence of a toxic or poisonous action of amniotic fluid. However, aspiration of amniotic sac contents, to which meconium has been added, gives an entirely different picture.

(Dr. E. T. Bell, Minneapolis.) It is remarkable how often one finds meconium in the alveoli in newborn infants. We had one case with a well developed purulent pneumonia in an infant who lived only 8 hours. There is no question that some of these cases are intra-uterine in origin.

(Dr. Edward C. Rosenow, Rochester, Minn.) I should like to ask if the matter of seasonal incidence has been considered. During experiments with streptococci of high virulence from cases of influenza it is not uncommon to find that organisms injected even intratracheally in pregnant animals will appear in the lungs of the fetuses. In view of the fact that normal persons become carriers of the streptococcus resembling in cataphoretic time and velocity and virulence the streptococcus of influenza in winter, it seems to me it would be interesting to determine whether or not there was a seasonal occurrence of pneumonia in newborn infants.

(Dr. Warwick, closing.) I agree that the question of amniotic fluid without meconium is of importance. It is hard to believe that just ordinary fluid causes pneumonia, but I find the great majority of my cases show meconium. That is why I took one series to show just meconium and the cornified epithelial cells.

In answer to Dr. Rosenow's question about seasonal incidence I do not know about that. I do know that these tended to occur in groups. Perhaps they had a seasonal incidence. I will look that up when I get back to Buffalo.

I did not have time to mention that two of the cases were in infants of only 5 months gestation, in which the alveoli were imperfectly formed, and the primitive alveoli were filled with polymorphonuclear leukocytes. In this case I was not able to demonstrate meconium with certainty, but there were granules there that looked like meconium, and I showed it to other pathologists who thought it might be meconium in which the bile salts did not have the color they would have later. I think practically all my cases showed meconium, but it varied in amount. I think perhaps a small amount of meconium might not cause a pneumonia, whereas a large amount would. Then, too, the condition of the infant might have something to do with it. If we had an infant who was subjected to

unusual procedures during birth or before, it might be more susceptible to the action of the amniotic fluid containing irritating material.

STUDIES IN PNEUMONIA OF INFANCY AND CHILDHOOD BY THE WHOLE LUNG SECTION METHOD. Irving Graef and (by invitation) Charles Hendee Smith and Elizabeth Torrey Andrews, New York, N. Y.

Abstract. Clearness in the pathological diagnosis of pneumonia of infancy suffers from a limitation of anatomical studies to small blocks of tissue. MacNeil and his coworkers studied whole lung paraffin sections, but did not separate the common varieties of pneumonia beyond bronchopneumonia and alveolar (lobar) pneumonia.

At Dr. Smith's suggestion we have sectioned whole lung blocks in paraffin thin enough (8 to 12 microns) to combine topographical and histological studies. We have studied the lungs of 96 infants, aged 1 week to 4½ years, by this method.

Eighteen showed no evidence of pneumonia. Four showed rare forms of pneumonia. The remaining 74 cases were classified anatomically as follows:

Lobar Pneumonia: Although frequently found during life in infancy and childhood, it is a rare lesion at autopsy on account of the low death rate. Five cases, 15 per cent of those sectioned, corresponded to the picture of lobar pneumonia as seen in adults, and showed diffuse hemorrhagic or fibrinopurulent alveolar exudate. Lymphangitis and septal involvement were found in all, pleurisy in 4 of the 5 cases, hemorrhages and abscesses in 3.

Lobular Pneumonia: We studied 16 cases showing as the characteristic lesion hemorrhagic or fibrinopurulent exudate in the alveoli of the lobule. Bronchiolar involvement is usually limited to those in affected lobules, where they show acute suppuration. There is irregularity in the distribution of lobular involvement and in the density of the exudate. This form is often confluent and tends to involve especially the lobules about the hilum. In 3 cases a whole lobe was involved, yielding a gross appearance of lobar pneumonia. Peripheral lobules are less often affected. Foreign material is regularly found within the bronchi. Pleuritis and lymphangitis are rare. Parenchymal and bronchiolar abscesses and hemorrhage are common.

Fifty-three cases show evidence of *bronchopneumonia*, which could be readily divided into two anatomical varieties. One of these is characterized by infiltration of the outer layers of the walls of the bronchi and bronchioles with mononuclear cells (histiocytes or fibroblasts and lymphocytes) and suppurative endobronchiolitis. Parenchymal involvement is limited to a few of the alveoli immediately adjacent to the bronchi, the exudate consisting of a few mononuclear cells and a little fibrin. This lesion may be discrete or confluent. The confluent form shows coalescence of more massive lesions, resulting in a picture resembling confluent sublobular or lobular consolidation due to collapse of the intervening intact parenchyma.

For the second variety we have used the term *broncholobular pneumonia*. There is characteristic mononuclear infiltration of the bronchial wall, extensive suppurative endobronchiolitis, and in addition a fibrinous or fibrinopurulent alveolar exudate involving whole lobules, in some cases a major part of the lobe.

The unmixed form comprised 21 cases, the mixed 32 cases, 1 of which showed only doubtful evidence of true lobular involvement. Both forms are disseminated; unilateral involvement was encountered only once. Varying degrees of bronchial involvement are common when various lobes are compared and in the

same lobe. Collapse occurred in all but 2 cases, both belonging in the unmixed group. It was so extensive in 2 cases as to simulate lobar consolidation.

Lymphangitis of the septa, pleuritis, abscess formation and hemorrhage are rare in pure bronchopneumonia, and frequent in bronchlobular pneumonia. Evidence of aspiration of foreign material is infrequent in both forms. Thrombosis was rarely encountered.

This analysis suggests certain simple mechanisms that may determine the type of lesion found. Abscesses and ruptured bronchioles occur far more frequently in bronchlobular pneumonia than in the pure bronchial form, suggesting that involvement of the lobule occurs by secondary extension from the affected bronchiole. Another mechanism may be endobronchiolar extension of the exudate or infectious agent to the collapsed lobule. Occasionally there is primary diffuse involvement of the wall of the bronchus, the septa, and the terminal lobules. Bronchlobular pneumonia appears to be a more severe or advanced stage than uncomplicated bronchopneumonia.

In lobular pneumonia aspiration appears to be the primary mode of involvement, occurring most frequently in feeble infants (all 16 cases were in newly born, debilitated, or congenitally defective infants; 11 of the 16 were under 6 weeks of age). Two mechanisms may account for the lesions. There may be infection of the lobules by aspirated infective material or local infection of an obstructed bronchiole with extension through or rupture of its walls and extension to the alveoli of the lobule.

Permanent hilum involvement in aspiration lobular forms and in the bronchlobular forms may be related to shallow breathing with minimal ventilation in this portion of the lung, especially in debilitated infants, thus promoting stasis.

Discussion

(Dr. George R. Callender, San Antonio.) I should like to ask whether any bacteriological studies were made in relation to the type of pneumonia.

(Dr. Francis G. Blake, New Haven.) Did any of these cases follow measles or other infections?

(Dr. Rufus Cole, New York City.) May I ask the age of the children who had the lobar type of pneumonia?

(Dr. Howard T. Karsner, Cleveland.) In continuation of Dr. Cole's question I should like to have Dr. Graef explain the criteria for the anatomical diagnosis of lobar pneumonia.

(Dr. E. T. Bell, Minneapolis.) This is an important demonstration in the study of the problem of pneumonia by the whole section method. As I understand it, these are sections of lantern slide size which are projected directly. This method should be of value in teaching.

(Dr. Blake.) While no one will deny that for practical clinical purposes it may be desirable to retain the term lobar pneumonia, as representing a fairly characteristic clinical picture, I think one hesitates to try and draw any sharp distinction between lobular pneumonia and lobar pneumonia, because of the fact that the picture of pneumococcal pneumonia as a whole, as I have tried to indicate in some of our experiments, runs the whole gamut from focal lobular pneumonia to a diffuse lobar pneumonia. Furthermore, in a great many cases coming to autopsy one frequently finds lobar consolidation in one lobe and lobular consolidation in another lobe in cases in which there was apparently only one organism concerned in the infection — the pneumococcus. While the dis-

tion has certain practical uses, it seems to me there is a certain danger in too sharp a differentiation which tends toward a departmentalization of our conceptions of infection with the pneumococcus. The reason why one wishes to know the age of the children who had a diffuse lobar consolidation is because of the theory, without supporting experimental evidence, that increased sensitiveness or allergy is responsible for a diffuse lobar consolidation, as contrasted with a focal lobular consolidation. I would call attention to the fact that the results of our studies in guinea pigs are quite inconsistent with the notion that a high degree of reactivity is related to a diffuse, rapidly spreading lobar pneumonia, because of the fact that our less sensitive animals presented a dermal lesion more nearly resembling a lobar pneumonia than our more sensitive animals, which presented a lesion more nearly resembling lobular pneumonia.

(Dr. Graef, closing.) In reply to Dr. Callender's question, bacteriological studies have been made in a large number of these cases, in life both by throat swabs and blood cultures, and also by postmortem cultures of the lesions in the lung, but we have as yet made no attempt to correlate these.

In answer to Dr. Blake's question with reference to measles, one case of bronchopneumonia followed measles.

In answer to Dr. Cole the age distribution of the lobar pneumonias was one infant 1 month old, and the others distributed unevenly through the first year.

In reply to Dr. Karsner's question about the criteria for the anatomical diagnosis of lobar pneumonia, this covers the entire question as to whether or not the term lobar is merely a quantitative expression of involvement of the lung. We have separated aspiration forms from primary infective lobular pneumonia. One of the distinguishing features of the aspiration forms is the regular demonstration of foreign material in the affected lung. Furthermore, it is encountered in a special group of infants, *i. e.*, the newly born, the debilitated, or the congenitally defective.

In primary infectious forms the lesion may be lobular, or it may occupy the major part of the lobe, primarily or by confluence. Occasionally, as in the cases shown on the screen, the difference in the nature of the exudate from field to field microscopically, from lobule to lobule, is much less marked than in advanced lobar pneumonia, as one usually sees it in adults.

In the particular child shown with lobar pneumonia the exudate within 48 hours after an abrupt onset of the disease was essentially an edema fluid in which microorganisms, red blood cells and leukocytes were seen in almost every field. Naturally I had to limit my demonstrated material to the method we used, but gross pictures have been compared in all cases with essential microscopic findings.

If I might go back to the discussion of Dr. Warwick's paper we are very anxious to separate the aspiration lobular pneumonia from primary infective lobular pneumonia which follows infectious diseases. Children who are unable to cough will very readily aspirate food material and vomitus, and they show the picture of lobular consolidation with prominent hilum involvement. Lobular involvement may be confluent occasionally to the extent of a lobe. Where there is definite evidence of aspirated material in the lungs, whether it is meconium, bile pigment or epidermal cells and amniotic fluid, unless there is the characteristic mononuclear cell infiltration in the outer layers of the walls of the bronchioles we feel the term lobular or sublobular pneumonia would be a safer description of the lesion than bronchopneumonia. It is true that the bronchial walls

are affected in aspiration pneumonia, but only in the affected lobule, which I think is quite different from the disseminated lesion of the bronchi following a respiratory infection.

ATELECTASIS OF THE NEWBORN. Sidney Farber and (by invitation) James L. Wilson, Boston, Mass.

Abstract. The following conclusions are based on experimental work and a pathological and clinical study of the lungs of several hundred full term and premature newborn infants. The lungs do not expand fully with the first breath. A few days are required before complete expansion is reached in the full term infant, and a period of 6 weeks or longer in the premature infant. Areas of previously unexpanded lung (initial atelectasis) can be differentiated from collapsed lung tissue which has once been expanded (resorption atelectasis) by observations on the size, shape, and position of the alveolar lining cells. The lungs of early premature infants differ from those of full term infants by the presence of areas of unexpandable lung tissue characterized by scattered masses of cells, identical in appearance with cells lining fully formed alveoli. No lumens are present. These are similar to masses of cells without alveolar formation in the lungs of a $3\frac{1}{2}$ months fetus.

Since atelectasis may be present normally in the full term infant for several days it should not be accepted as a complete and satisfactory cause of death in the first few days of life. We have shown by measurement of the resistance to expansion of the lungs of dead infants, and dead and living animals, that the cohesion of the moist surfaces of the respiratory passages in collapsed lungs offers a considerable resistance to the entrance of air and that a relatively great force is required to overcome this cohesion and to separate the air passages during the initial expansion of an atelectatic lung. The very first breath of every newborn infant may thus be its most difficult one, and therefore, for a variable period after birth, especially vigorous inspirations must be maintained until atelectasis finally disappears. This factor is always present in the newborn infant. It may be combined with any of the following conditions, in explanation of the pathogenesis of atelectasis of the newborn:

1. An imperfectly developed or an injured respiratory center (hemorrhage).
2. An imperfectly developed thoracic mechanism (premature infants) when the contraction of the intercostal muscles and the bony resistance of the thorax do not furnish an effective *vis a tergo* for the efficient action of the diaphragm.
3. Bronchial obstruction due to aspiration of amniotic sac contents, mucus or blood.

Discussion

(Dr. Rafael Dominguez, Cleveland.) I should like to call the attention of Dr. Farber to the fact that the initial response to deformation in organic elastic bodies is well known. It has been known since the experiments of Weber, from Göttingen, on the elastic properties of silk threads, which require a preliminary preparation before the elasticity can be quantitatively studied. This property has been found in many colloidal substances. It is familiar in the case of chewing gum, and it forms an important development in the physical literature, under the name of thixotropy.

THE SHWARTZMAN PHENOMENON IN THE LUNGS OF RABBITS. John Cohen (by invitation), New York City.

Abstract. In a study of the pathogenicity of the organisms of human lung abscess there is encountered a group of non-chromogenic, strictly anerobic, gas-producing Gram-negative bacilli with foul odor which elicit a severe gangrenous bronchopneumonia and empyema in the lungs of rabbits when injected intratracheally. This ability is only shared by these organisms, for the other organisms of lung abscess do not produce these lesions (*i. e.*, cultures of streptococci, diphtheroids, *B. ramosus*, *B. fusiformis* and other aerobic organisms, singly or in combination). Rabbits dying spontaneously in the laboratory and examined routinely show a hemorrhagic lobar pneumonia which differs entirely from the gangrenous type produced.

We then attenuated the virulence of these organisms in different culture media so that mild lesions (patchy hemorrhagic bronchopneumonia without necrosis) were produced in the lungs of rabbits or none at all. If an organism of such diminished virulence was injected intratracheally and a mild reaction induced in the lungs of rabbits, and then in 24 hours the homologous culture filtrate was injected intravenously, the lesions in the lungs appeared at a maximum, namely, gangrenous bronchopneumonia and empyema, thus eliciting the Shwartzman phenomenon in the lungs. This phenomenon has been previously demonstrated by the use of sterile filtrates in the kidney and liver (Shwartzman), in the stomach (Karsner, Ecker and Jackson), in the knee joints (Moritz and Morley), and in the intestinal tract (Gratia and Linz).

Discussion

(Dr. Howard T. Karsner, Cleveland.) The discovery of the Shwartzman phenomenon is an extremely important contribution. In its simplest terms it appears to be essentially due to vascular injury. In Dr. Cohen's communication it is apparent that the primary dose of filtrate had to be adjusted delicately so that the injury was comparatively slight. The later injection augmented the vascular injury, as indicated by the hemorrhage and thrombosis. Is there a specific relation between the human pulmonary disease, from which Dr. Cohen isolated the organisms, and that produced in the lungs of the experimental animals? In other words, is it not possible that filtrates of other organisms used in similar fashion produce essentially the same lesion?

(Dr. Francis G. Blake, New Haven.) I think one has to take exception to the statement that other organisms have not satisfactorily produced lung abscesses in experimental animals. Certainly there is no difficulty in experimentally producing typical necrosis and lung abscess in monkeys with pure cultures of hemolytic streptococci.

(Dr. Cohen, closing.) In answer to Dr. Karsner, by injecting the toxin or the toxic filtrate intratracheally into rabbits, and then injecting the toxic filtrate intravenously, no lesions in the lungs are obtained. It appears that unless a mild lesion in the lung is produced with a live and attenuated organism the phenomenon cannot be obtained after the intravenous injection. We have not studied any other organism, because for the present we have paid undivided attention to the non-chromogenic, strictly anerobic, Gram-negative bacillus, which has proved uniformly pathogenic.

In reply to Dr. Blake, we have not found at any time that injection of the anaerobic streptococcus into the lungs of rabbits produced any lesion. The *Streptococcus putridus* (Schottmüller), which produces hemolysis on blood plates and gas, and foul odor on suitable media, will also produce a hemorrhagic pneumonia, but not of the type of gangrene associated with this Gram-negative anaerobic bacillus.

IMMEDIATE PNEUMOCOCCUS TYPING DIRECTLY FROM SPUTUM BY THE NEUFELD REACTION. Albert B. Sabin (by invitation), New York City.

Abstract. Neufeld observed that when pneumococci are mixed with specific immune serum there occurs in addition to agglutination a *Quellung* (swelling) of the peripheral zone of the organisms. Early attempts to apply this observation to the typing of pneumococci in sputum were not uniformly successful for various reasons. A study of the various factors involved revealed the following to be of great importance: (1) The horse antipneumococcus serums commonly used for typing were unsuitable for this method because they frequently gave non-specific reactions, which could not be satisfactorily eliminated by dilution; (2) carefully prepared rabbit serum gives absolutely specific results; (3) the preparation must be observed in the moist state, and alkaline methylene blue aids in visualization.

The following method was successfully employed with sputum from 100 cases of lobar pneumonia. To a small fleck of sputum a small loopful of rabbit serum and a loopful of alkaline methylene blue are added. This preparation (hanging drop) is immediately observed with the oil immersion lens. When the organism is of the same type as the serum a characteristic zone of ground glass appearance is seen around it. This method was successful in every case; it dispenses with the use of mice and permits a correct determination of pneumococcus type directly from the sputum within a few minutes.

Discussion

(Dr. Roderick Heffron, by invitation, Boston.) I should like to say a word about this method of typing. At the State Bacteriological Laboratory in Boston we have been using the Neufeld method of typing along with the Krumwiede, Sabin and tube agglutination methods for about 6 months. During the past 4 months the Neufeld method has been used routinely.

For this method of typing we have used the technique kindly demonstrated to us by Dr. Kenneth Goodner. Recently we have altered this to the extent of using flat slide preparations instead of the original hanging drop method.

Pneumococcus typing of any sort is preferably carried out on fresh material; however, the majority of our specimens come by mail and are, on the average, from 12 to 24 hours old when received. Despite the age of such specimens we have had extremely gratifying typing results using the Neufeld technique.

During the past 6 months sputums from 109 cases of pneumonia of various sorts have been typed by this method. Forty of these were correctly diagnosed as Types I, II, or III. The results in two others were questionable; later these were found to be of the type first suspected.

Of the 109 cases typed by this method results (for Types I, II, or III) were negative in only 3, which later proved to be one of these types. In each of these instances no pneumococci were seen in the original preparations, but they were

isolated following mouse inoculation. So far it has been possible to check the results of every Neufeld typing by at least one other method.

In Massachusetts, as elsewhere, time is a most important factor in the successful serum treatment of lobar pneumonia. During the brief period the Neufeld technique has been used we have been able to save as much as 12 to 24 hours on our reports in many instances.

The method has also proved valuable for typing broth cultures, cultures on solid media, and mouse exudates where the flora is mixed and pneumococci are few. In the latter case the Neufeld technique has yielded positive results when other methods were not applicable.

If, after further study, we continue to find the Neufeld method as reliable as it now appears, the procedure will be taught to our collaborating technicians throughout the state. Rabbit typing serum will be supplied to them in order that the method may be routinely used.

(Dr. Frank B. Lynch, Philadelphia.) It seems to me from the appearance of these slides that there might be some confusion in the appearance of these organisms with that of the Type III pneumococcus. I should like to ask if there has been any difficulty in that respect.

(Dr. Sabin, closing.) In response to Dr. Lynch, I can say that among the 100 cases there were 4 of Type III, and in no instance, in the hanging drop preparation, did the Type III organism show a capsule.

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